

Chapter 2

Viral Gene Therapy Vectors

Abstract The vectors for gene delivery can be divided into two classes: viral and nonviral. Despite the rapid progress in the development of nonviral gene delivery, viral vectors such as retrovirus/lentivirus, adenovirus, adeno-associated virus (AAV) and baculovirus mediate more efficient delivery than nonviral vectors, especially for primary cells. This chapter briefly compares the nonviral and viral vectors and mainly discusses the development and characteristics of these viral vectors.

2.1 Gene Therapy

Gene therapy is a technique originally developed to deliver DNA or RNA molecules to cells/tissues for the treatment of genetic diseases. Nonetheless, gene therapy has been rapidly expanded to a wide variety of applications such as treatment of cancers and infectious diseases. For example, delivery of Diphtheria toxin A gene via baculovirus inhibits the glioma xenograft growth in the rat brain [1] and delivery of the hemagglutinin gene of avian influenza virus to mice and poultry via adenovirus elicits immunity and confers protection [2]. Gene therapy and tissue engineering have also converged for the repair of various tissues/organs, such as musculoskeletal and cardiovascular systems. For instance, gene therapy in conjunction with tissue engineering can aid in the treatment of myocardial infarction [3, 4], bone defects [5] and diseases in cartilage [6–8]. Although protein-based therapy also provides an effective approach for the treatment of bone/cartilage defects, it might be ineffective in repairing large defects clinically [9]. In the case of bone repair, a single protein (e.g. bone morphogenetic protein 2 (BMP-2)) dose may not confer an adequate

repair response because of the short protein half-life and poor retention in large defects [10], thus milligrams of proteins or multiple doses are required. With this regard, gene therapy offers an attractive option to augment tissue repair [10]. Since the gene, rather than a degradable protein, is being delivered, gene delivery potentially results in higher and more sustained protein release in a more physiologic manner than recombinant protein therapy [10]. Moreover, the endogenously synthesized proteins may have greater biological effectiveness than their exogenous counterpart.

Gene therapy can be performed either *in vivo* or *ex vivo*. The *in vivo* gene delivery involves the injection or implantation of genetic material carried by the delivery vector directly into the host. This approach is simpler and minimizes the risk of infection since only one procedure is required [11]. However, direct vector injection, either locally or systemically, may elicit inflammatory responses which interfere with the reparative process [12]. It is also extremely difficult to specifically deliver the genes into target cells *in vivo*, thus resulting in low levels of protein expression. In the context of tissue engineering, typically the vector is administered locally to minimize unwanted side effects, but it is difficult to avoid the transgene expression in secondary tissues. Another challenge is how to achieve a sustained long-term expression of the therapeutic gene, although in some cases a short-term expression is sufficient to accelerate healing of tissues and may be desirable [10].

Ex vivo gene delivery involves the genetic modification of cells (either autogenic or allogenic) and re-introduction into the host with or without a scaffold. The *ex vivo* strategy enhances the roles of the cells in the regenerative process with autocrine/paracrine effects from the expressed transgene products. In one *ex vivo* gene therapy study, the articular chondrocytes are genetically modified by transfection of cDNA encoding insulin-like growth factor 1 (IGF-1), encapsulated and implanted into osteochondral defects in rabbits. The genetic modification results in prolonged IGF-1 expression *in vitro* (up to 36 days), augments articular cartilage repair and accelerates the formation of subchondral bone [13]. The disadvantage of such *ex vivo* therapy in the tissue engineering setting is that it involves two separate invasive procedures for a patient (when autologous cells are used), which increases the pain the patients suffer and possibility of morbidity. Moreover, the cells transplanted into the defects may not persist for a period of time sufficient to heal the defects [14].

2.2 Vectors for Gene Delivery

2.2.1 *Nonviral vs. Viral Vectors*

The vectors for gene delivery can be divided into two classes: viral and nonviral. Nonviral vectors mainly rely on the delivery of plasmid DNA (or other forms of DNA/RNA) into cells/tissues with the aid of a proper transfection reagent (e.g. liposomes, polymer-based molecular conjugates, nanoparticles, etc). The nonviral vector carrying sex-determining region Y box gene 9 (SOX-9) gene has been shown to enhance the

chondrogenesis of mouse bone marrow-derived mesenchymal stem cells (BMSCs) [15]. Transfection of the DNA encoding IGF-1 into articular chondrocytes and transplantation of transfected cells also lead to the formation of a new tissue layer on the cartilage explant surface [16]. In addition, primary chondrocytes and explants can be engineered by transfection of DNA encoding human glycosaminoglycan (GAG)-synthesizing enzyme, β 1,3-glucuronosyltransferase-I (GlcAT-1). Such GlcAT-1 delivery enhances the glycosaminoglycan (GAG) deposition and overcomes interleukin 1 β (IL-1 β)-induced proteoglycan depletion [17].

Despite the rapid progress in the development of nonviral gene delivery vector, transfection methods (e.g. in vivo electroporation [18], microporation [19], nucleofection [20]) and transfection reagents (e.g. FuGENE6 [21], nanoparticles [22, 23]) to enhance the efficiency of transfection into cells, it is generally perceived that the efficiency of gene delivery mediated by nonviral vectors is lower when compared with that by viral vectors [24–26]. In particular, transfection of adult MSCs is very inefficient [27].

In contrast, viral vectors are widely employed for gene therapy as viruses naturally evolve mechanisms for effective delivery of their genetic cargo into cells for replication and expression [28]. For vector development, generally the elements of viral genome that contribute to replication, virulence and pathology are deleted and replaced by gene(s) of interest while retaining the elements contributing to efficient delivery [24, 29]. The viral vectors that are in common use include retrovirus/lentivirus, adenovirus and adeno-associated virus (AAV) [25, 26]. Some emerging viral vectors such as baculovirus have also been investigated (for review, see [30–35]). Therefore, this chapter mainly deals with these viral vectors.

2.2.2 *Retrovirus/Lentivirus*

Retrovirus genome comprises two identical RNA molecules, which after entry into the cells are reverse transcribed to complementary DNA and integrate into the host chromosome. The integration ensures the persistence of the therapeutic gene in the cells, thus retrovirus is initially favored for applications whereby long-term expression is desired. In fact, retrovirus is the viral vector used for the first human gene therapy clinical trial for the treatment of a genetic disease known as severe combined immunodeficiency (SCID) [36]. In this clinical trial, a gene encoding adenosine deaminase (ADA) is transduced into autologous T cells using the retroviral vector and delivered into two patients. Four years after the initial treatment, the ADA expression is still detectable and the symptoms are alleviated in one patient, thus demonstrating the proof-of concept of gene therapy [36].

The first and the most commonly used retrovirus is Molony murine leukemia virus (MuLV). MuLV transduces synovial fibroblasts cultured in vitro with reasonable efficiency, but is inactive in in vivo experiments when injected into the knee joints. The inefficiency is partly due to the fact that retrovirus only transduces proliferating cells, but not quiescent cells. Besides, the extracellular matrix (ECM)

hinders retrovirus from directly transducing chondrocytes embedded in the ECM. Therefore, retrovirus is more suitable for ex vivo gene transfer-mediated tissue engineering. Retrovirus expressing enhanced green fluorescent protein (EGFP) is used to transduce chondrocytes, followed by implantation of the transduced cells into full-thickness defects in knee joints of rabbits [37]. The EGFP expression and the number of implanted chondrocytes remain stable for at least 4 weeks in vivo.

Note, however, that the integration does not guarantee long-term transgene expression because (1) the genes might be silenced as a result of epigenetic modification [38] and (2) the transduced cells may be eradicated due to regular turnover or the immune system. Retrovirus-mediated transfer to autologous synoviocytes results in transgene expression that steadily diminishes over a period of 4–6 weeks following intra-articular implantation of the transduced cells [39].

Moreover, retrovirus transduces synoviocytes in the inflamed joints better than those in the naïve joints [8]. Since one of the primary symptoms of rheumatoid arthritis (RA) is the thickening of the synovium through synovial cell proliferation, the study implicates the application of retroviral vectors in gene delivery to the joints of RA patients [8]. Retroviral vector-based gene therapy for RA treatment has entered clinical trial [6], in which the retrovirus expressing interleukin 1 (IL-1) receptor antagonist (IL-1Ra) is used to transduce autologous synovial cells ex vivo, followed by implantation of the transduced cells into metacarpophalangeal joints of RA patients 1 week prior to the scheduled joint replacement surgery. No adverse effects related to the gene transfer are observed and there is no relevant spread of the transgene to extra-articular sites [6].

However, retrovirus preferentially integrates viral genes to the transcription start sites and highly expressed genes in the host chromosome, thus raising serious safety concerns. Maria Cavazzana-Calvo, Alain Fischer and coworkers have demonstrated the use of retroviral vectors for the cure of X-linked SCID in nine out ten patients [40]. Unfortunately, two of the patients develop leukemia as retrovirus integrates into chromosomal sites in proximity to the LMO-2 proto-oncogene promoter, leading to aberrant expression of LMO2 [41]. Follow-up studies have cured more than 20 patients and confirmed the efficacy of SCID gene therapy, but leukemia occurs in several more patients [42–44]. Furthermore, the in vivo application of retrovirus in humans is compromised by their sensitivity to inactivation by the complement system [45].

Lentivirus belongs to the retrovirus family but is different in that lentivirus has a more complex genome and is capable of transducing non-dividing cells. Similar to other retroviruses, lentivirus has a low DNA carrying capacity of ≈ 8 kb and can stably integrate the viral genes into the host genome. The transduction spectrum of lentivirus can be broadened by pseudotyping the virus envelope with VSV-G (vesicular stomatitis virus G protein). Such VSVG-pseudotyped lentivirus can transduce cultured chondrocytes and mediate gene transfer to synovium [46]. Lentivirus expressing SOX-9 also enhances collagen II expression and down-regulates the collagen I expression of passaged chondrocytes, implicating the potential of lentivirus-mediated SOX-9 expression in restoring chondrocyte phenotype even after de-differentiation [47]. Lentivirus is also used for intra-articular delivery of

endostatin [48] and angiostatin [49] into rodents to treat experimental models of RA. To date, use of lentivirus for ex vivo transduction of CD34⁺ cells has entered into clinical trials. One example is the use of lentiviral vector for the treatment of patients with X-linked adrenoleukodystrophy (ALD). The trial confirms that lentivirus-mediated gene therapy provides clinical benefits in ALD [50].

However, lentiviral vectors (e.g. the vector derived from human immunodeficiency virus (HIV)) also favor the integration into active transcription units [51]. The integration may elicit insertional mutagenesis [52, 53] and raise safety concerns. To circumvent these problems, non-integrating lentiviral vectors have been developed [54]. These lentiviral vectors are defective in integrase (the enzyme responsible for integration), thus enabling the maintenance of transgene in the episomal form while conferring stable transgene expression [55].

Unlike the popular use of retrovirus/lentivirus in other applications requiring long-term expression (e.g. treatment of inherited diseases), whether retroviral/lentiviral vectors will be deemed safe for clinical use in bone/cartilage tissue engineering is questionable. Justification of the safe use of retroviral/lentiviral vectors in bone/cartilage regeneration requires further testing.

2.2.3 Adenovirus

Adenovirus has a 36 kb, double stranded DNA genome packaged in a 100 nm icosahedral capsid. Wild-type adenovirus infects cells in the upper respiratory tract and can result in mild cold. Adenovirus is able to infect dividing and nondividing cells, which provides advantages for in vivo gene delivery (for review, see [56]). There are more than 50 adenovirus serotypes and initially emphasis is placed on serotype 5 (Ad5). Nonetheless, more and more adenovirus serotypes are explored for gene therapy.

The first generation adenoviral vectors are deleted in E1 and E3 genes, which allows for the insertion of up to 8 kb of foreign gene cassette. Adenovirus can be produced to high titers, which renders the vector production simpler and more cost-effective. Adenovirus genome does not integrate at high efficiency and remains episomal, thus the viral genomes only persist in non-dividing cells and the therapeutic gene expression is lost when the transduced cells are gradually diluted out of the population.

The adenovirus expressing the *lacZ* gene is employed for in vivo gene delivery to joints, which results in transgene expression for over a month within the synovium without provoking an inflammatory response [57]. However, inflammatory responses are found in subsequent studies exploiting adenovirus-mediated delivery of p53 [58], IL-1 and tumor necrosis factor α (TNF- α) receptors [59] to disease joints, causing the rapid decline and extinguishing of transgene expression in 2–4 weeks. The transient expression results from the strong cellular immune responses elicited by the continued expression of endogenous viral proteins within the cells.

Nonetheless, adenoviral vector is widely used for gene therapy in the context of tissue engineering. In particular, the adenovirus-mediated IGF-1 expression can last for at least 28 days and effectively enhances *in vitro* chondrogenesis [60]. Adenovirus-mediated IGF-1, transforming growth factor β 1 (TGF- β 1) and BMP-2 expression in chondrocytes greatly increases matrix synthesis *in vitro*, even in the presence of the inflammatory cytokine IL-1 [61]. Furthermore, two adenoviral vectors expressing IGF-1 and IL-1Ra are used in combination to co-transduce cultured synoviocytes. The IGF-1 and IL-1Ra secreted by the transduced cells fully reverse the depletion of cartilage proteoglycan contents induced by IL-1 [62].

The biggest barrier to the clinical application of adenovirus is the strong humoral and cellular immune responses it elicits, especially after the tragedy death in an adenovirus-mediated gene therapy trial [63]. To minimize the immune responses, the “gutless” vectors that contain only the viral terminal repeats and the packaging sequence are developed [64]. In the gutless vector, all other viral components are deleted, thus it can accommodate up to 36 kb of exogenous DNA and does not trigger strong immune responses. However, gutless adenovirus vector, due to the deletion of most viral components, requires helper plasmid or virus for production, making the production process more complicated. Furthermore, the transgene expression appears to be weakened [65]. Additionally, most humans have pre-existing immunity to adenovirus which could neutralize the administered virus vectors. Such pre-existing immunity problem may be circumvented by using adenoviral vectors derived from different serotypes.

2.2.4 AAV

AAV is a parvovirus with a \approx 4,700 nt, single-stranded DNA genome (for review, see [66]). The genome replication of AAV requires helper viruses such as adenovirus or herpes simplex virus to provide helper functions. AAV alone is non-pathogenic to humans and does not induce serious host immune responses. AAV can mediate long-term transgene expression in a wide variety of cells, including dividing and non-dividing cells. These advantages have inspired the wide application of recombinant AAV vectors for gene delivery [67]. Like adenovirus, AAV exists in many serotypes, among them AAV 2 and AAV 5 are most extensively studied and utilized.

The feasibility of utilizing AAV vector in tissue engineering has been demonstrated by a study in which AAV is used for direct *in vivo* modification of synoviocytes and the β -galactosidase expression in the synovium is observed for at least 7 months [68]. A subsequent study also shows efficient (transduction efficiency >70 %) and persistent recombinant AAV vector transduction of chondrocytes derived from normal and osteoarthritic human articular cartilage [69]. Strikingly, transduction of explant cultures of articular cartilage results in reporter gene expression within the tissue to a depth exceeding 450 μ m, which remains persistent for 150 days [69]. These data suggest that AAV vectors are able to transduce chondrocytes *in situ* within their native matrix to a depth sufficient to be of important clinical significance [69].

Furthermore, AAV-mediated delivery of TGF- β 1 gene improves the expression of collagen II and aggrecan while decreases the matrix metalloproteinase 3 (MMP-3) expression in cultured normal and osteoarthritic chondrocytes [70]. In vitro transduction of chondrocytes with an AAV vector expressing fibroblast growth factor (FGF-2) stimulates cell proliferation over a long period of time, and in vivo application of the same AAV vector significantly improves the overall repair of osteochondral defects in rabbit knee joints [11]. AAV vector is also used to deliver the gene encoding basic fibroblast growth factor (bFGF) into articular chondrocytes [71]. The transduced autologous cells are embedded into collagen gels and re-implanted into a full-thickness defect in the articular cartilage of the rabbit patellar groove. The transduction leads to the expression exceeding 8 weeks in >85 % of in vitro population and leads to the repair of articular cartilage defect [71]. AAV expressing receptor activator of nuclear factor κ B ligand (RANKL) and vascular endothelial growth factor (VEGF) can also be freeze-dried into the allograft bone. Implantation of the coated allografts leads to marked formation of a new bone collar around the graft [72].

Currently, AAV appears to be the most promising vector for gene therapy, and may offer the best compromise between safety and efficacy for in vivo gene transfer [28, 65]. In 2012, Glybera®, an AAV vector designed to treat lipoprotein lipase deficiency, becomes the first gene therapy product approved in the Western world [73]. Additionally, an AAV vector expressing the TNF antagonist is employed in phase I and II clinical trials aiming for the treatment of rheumatoid arthritis (RA) [74]. The gene product is identical to etanercept (Enbrel®) used to treat RA patients and blocks the actions of TNF [75]. Intra-articular injection of this AAV vector exerts symptomatic benefit in some patients [74]. Although one subject dies in 2007 during the trial, the death is not attributed to AAV [76].

One challenge to the clinical application of AAV is that a large portion of human population possesses neutralizing antibodies against AAV [77], which diminishes the in vivo efficacy of AAV. Furthermore, the transduction efficiency of AAV vectors is hindered by the requirement to convert the single stranded DNA genome into double stranded DNA prior to expression. This rate-limiting step prompts the development of self-complementary AAV (scAAV) vectors, which package an inverted repeat genome that can fold into double stranded DNA and can increase the transduction efficiency [78]. The trade-off of such scAAV is the loss of half of the cloning capacity.

Another challenge for the clinical application of AAV is the difficulties and high cost associated with production of high titer AAV, which requires transfection of producer cells with multiple plasmids. To overcome this problem, the genes required for AAV production can be cloned into separate baculovirus vectors (see Sect. 2.2.5), which, after co-infection of insect cells lead to the expression of AAV proteins and assembly of recombinant AAV vectors [79]. The new baculovirus/insect cell-based AAV production method is exploited for the production of Glybera®, the sole approved gene therapy product, and may encourage wider applications of AAV vectors in gene therapy.

2.2.5 *Baculovirus*

Baculoviruses are a diverse group of DNA viruses capable of infecting more than 500 insect species. Among the numerous baculoviruses, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) contains a circular double-stranded DNA genome of ≈ 134 kb and is the most widely used. Budded AcMNPV is highly infectious to cultured insect cells, thus recombinant baculoviruses have been engineered to carry exogenous genes to infect insect cells for the production of numerous recombinant proteins (for review, see [80, 81]). Since the finding that baculovirus can efficiently transduce mammalian cells in the mid-1990s [82, 83], numerous permissive cells from different species have been discovered (for review, see [84–86]). Baculovirus neither replicates nor is toxic inside the transduced mammalian cells [84]. Baculoviral DNA degrades in the cells over time [87, 88] and there is no evidence of baculoviral DNA integration into host chromosomes unless selective pressure is applied [89]. These attributes minimize the potential side effects and ease the safety concerns. Furthermore, the large baculovirus genome confers a huge cloning capacity of at least 38 kb [90] and baculovirus can be propagated to high titers easily by infecting its natural host insect cells [86]. These properties have fueled growing interests to explore baculovirus for a wide variety of applications, ranging from protein production [91, 92], virus production [93–95], virus-like particle production [88, 96, 97], eukaryotic protein display [98, 99], vaccine development [100–102], cancer therapy [103] to cell-based assay development [104–106].

Importantly, baculovirus is able to transduce primary chondrocytes derived from rats [87] and rabbits [107] with efficiencies exceeding 80 %. Baculovirus transduction does not hamper normal chondrocyte differentiation. Furthermore, baculovirus transduces bone marrow-derived mesenchymal stem cells (BMSCs) [108], adipose-derived stem cells (ASCs) [109] and even cell sheets derived from ASCs [110]. Under optimized conditions, the transduction efficiencies can exceed 95 %. Baculovirus also transduces adipogenic, chondrogenic and osteogenic progenitors originating from human BMSCs without obstructing the proliferation and differentiation potentials [111]. These properties spark the interests to develop baculovirus as a vector for the tissue engineering of bone, cartilage [112] and heart [110].

One shortcoming of baculovirus, however, is that baculovirus typically mediates transient (<7 days) transgene expression due to its non-replicating nature. Such transient expression may preclude the applications of baculovirus in certain scenarios requiring long-term sustained transgene expression (e.g. cancer therapy). To prolong the expression, attempts to incorporate AAV inverted terminal repeats [113, 114] or *Sleeping Beauty* transposon [115, 116] into baculovirus vectors have been made. For instance, a hybrid baculovirus exploiting the *Sleeping Beauty* transposon system is developed to extend the expression of microRNA [115] and anti-angiogenic factors for anti-cancer therapy [116].

Aside from these baculovirus vectors relying on transgene integration, hybrid baculovirus vectors enabling the episomal maintenance of transgene have been designed. We develop a hybrid dual baculovirus system in which one baculovirus

expresses FLP recombinase while the substrate baculovirus harbors the transgene cassette flanked by two Frt sequences [108]. After co-transduction of mammalian cells with the two baculovirus vectors, the expressed FLP recognizes the Frt sites and excises the Frt-flanking cassette off the baculovirus genome, and hence catalyzes the recombination and formation of episomal DNA minicircles encompassing the transgene cassette. Such hybrid baculovirus vector successfully extends the transgene expression in a number of mammalian cells, including rabbit BMSCs [108] and ASCs [117]. The expression level and duration positively correlate with the recombination efficiency, presumably because the smaller DNA minicircle are less prone to nuclease attack and gene silencing [118, 119].

The excision/recombination efficiency is remarkably high, reaching 75 % in HEK293 cells, 85 % in BHK cells and 77 % in primary chondrocytes [108]. However, the FLP/Frt-mediated recombination efficiency occurs in only ≈ 40 –50 % of rabbit BMSCs and ASCs [117, 120]. To further enhance the recombination efficiency, we have explored the codon-optimized FLP (FLPo), which can improve the FLP/Frt-mediated recombination at 37 °C [121]. Additionally, two other site-specific recombinases, Cre and codon-optimized Φ C31 (Φ C31o), have been tested. Φ C31o mediates excision/recombination between the heterotypic sites *attP* and *attB*, while Cre catalyzes excision/recombination events between two identical loxP sites [122]. Similar to the FLP/Frt-based baculovirus system, we construct a binary baculovirus vector system. Upon co-transduction, the transgene in the substrate baculovirus is excised by the recombinase (Φ C31o, Cre or FLPo) expressed by a second baculovirus vector and recombines into the smaller minicircle [123]. The recombination efficiency is lower by Φ C31o (≈ 40 –75 %), but approaches ≈ 90 –95 % by Cre and FLPo in various cell lines and stem cells such as human ASCs [123]. Compared with FLPo, Cre exerts higher expression level and lower cytotoxicity in human ASCs. The Cre/loxP-based baculovirus vectors are used to deliver genes encoding BMP-2 or VEGF into human ASCs, which results in efficient Cre/LoxP-mediated recombination and minicircle formation. As a result, the growth factor (BMP-2 or VEGF) expression is significantly prolonged and enhanced in human ASCs. The prolonged BMP2 expression ameliorates the osteogenesis of human ASCs, a stem cell with poor osteogenesis potential [123].

References

1. Wang C-Y, Li F, Yang Y, Guo H-Y, Wu C-X, Wang S (2006) Recombinant baculovirus containing the Diphtheria toxin A gene for malignant glioma therapy. *Cancer Res* 66:5798–5806
2. Gao WT, Soloff AC, Lu XH, Montecalvo A, Nguyen DC, Matsuoka Y et al (2006) Protection of mice and poultry from lethal H5N1 avian influenza virus through adenovirus-based immunization. *J Virol* 80:1959–1964
3. Siltanen A, Kitabayashi K, Patila T, Ono M, Tikkanen I, Sawa Y et al (2011) Bcl-2 improves myoblast sheet therapy in rat chronic heart failure. *Tissue Eng Part A* 17:115–125
4. Siltanen A, Kitabayashi K, Lakkisto P, Makela J, Patila T, Ono M et al (2011) hHGF overexpression in myoblast sheets enhances their angiogenic potential in rat chronic heart failure. *PLoS One* 6:e19161

5. Rundle CH, Miyakoshi N, Kasukawa Y, Chen ST, Sheng MHC, Wergedal JE et al (2003) In vivo bone formation in fracture repair induced by direct retroviral-based gene therapy with bone morphogenetic protein-4. *Bone* 32:591–601
6. Evans CH, Ghivizzani SC, Herndon JH, Wasko MC, Reinecke J, Wehling P et al (2000) Clinical trials in the gene therapy of arthritis. *Clin Orthop* 379:S300–S307
7. Evans CH, Gouze JN, Gouze E, Robbins PD, Ghivizzani SC (2004) Osteoarthritis gene therapy. *Gene Ther* 11:379–389
8. Ghivizzani SC, Lechman ER, Tio C, Mule KM, Chada S, McCormack JE et al (1997) Direct retrovirus-mediated gene transfer to the synovium of the rabbit knee: implications for arthritis gene therapy. *Gene Ther* 4:977–982
9. Hsu WK, Sugiyama O, Park SH, Conduah A, Feeley BT, Liu NQ et al (2007) Lentiviral-mediated BMP-2 gene transfer enhances healing of segmental femoral defects in rats. *Bone* 40:931–938
10. Lieberman JR, Ghivizzani SC, Evans CH (2002) Gene transfer approaches to the healing of bone and cartilage. *Mol Ther* 6:141–147
11. Cucchiariini M, Madry H, Ma C, Thurn T, Zurakowski D, Menger MD et al (2005) Improved tissue repair in articular cartilage defects in vivo by rAAV-mediated overexpression of human fibroblast growth factor 2. *Mol Ther* 12:229–238
12. Gelse K, Schneider H (2006) Ex vivo gene therapy approaches to cartilage repair. *Adv Drug Deliv Rev* 58:259–284
13. Madry H, Kaul G, Cucchiariini M, Stein U, Zurakowski D, Remberger K et al (2005) Enhanced repair of articular cartilage defects in vivo by transplanted chondrocytes overexpressing insulin-like growth factor I (IGF-I). *Gene Ther* 12:1171–1179
14. Mierisch CM, Wilson HA, Turner MA, Milbrandt TA, Berthoux L, Hammarskjöld ML et al (2003) Chondrocyte transplantation into articular cartilage defects with use of calcium alginate: the fate of the cells. *J Bone Joint Surg Am* 85A:1757–1767
15. Tsuchiya H, Kitoh H, Sugiura F, Ishiguro N (2003) Chondrogenesis enhanced by overexpression of sox9 gene in mouse bone marrow-derived mesenchymal stem cells. *Biochem Biophys Res Commun* 301:338–343
16. Madry H, Zurakowski D, Trippel SB (2001) Overexpression of human insulin-like growth factor-I promotes new tissue formation in an ex vivo model of articular chondrocyte transplantation. *Gene Ther* 8:1443–1449
17. Venkatesan N, Barre L, Benani A, Netter P, Magdalou J, Fournel-Gigleux S et al (2004) Stimulation of proteoglycan synthesis by glucuronosyltransferase-1 gene delivery: a strategy to promote cartilage repair. *Proc Natl Acad Sci U S A* 101:18087–18092
18. Kimelman-Bleich N, Pelled G, Zilberman Y, Kallai I, Mizrahi O, Tawackoli W et al (2011) Targeted gene-and-host progenitor cell therapy for nonunion bone fracture repair. *Mol Ther* 19:53–59
19. Wang YH, Ho ML, Chang JK, Chu HC, Lai SC, Wang GJ (2009) Microporation is a valuable transfection method for gene expression in human adipose tissue-derived stem cells. *Mol Ther* 17:302–308
20. Aslan H, Zilberman Y, Arbely V, Sheyn D, Matan Y, Liebergall M et al (2006) Nucleofection-based ex vivo nonviral gene delivery to human stem cells as a platform for tissue regeneration. *Tissue Eng* 12:877–889
21. Dinser R, Kreppel F, Zaucke F, Blank C, Paulsson M, Kochanek S et al (2001) Comparison of long-term transgene expression after non-viral and adenoviral gene transfer into primary articular chondrocytes. *Histochem Cell Biol* 116:69–77
22. Park JS, Na K, Woo DG, Yang HN, Kim JM, Kim JH et al (2010) Non-viral gene delivery of DNA polyplexed with nanoparticles transfected into human mesenchymal stem cells. *Biomaterials* 31:124–132
23. Kim T-H, Kim M, Eltohamy M, Yun Y-R, Jang J-H, Kim H-W (2013) Efficacy of mesoporous silica nanoparticles in delivering BMP-2 plasmid DNA for in vitro osteogenic stimulation of mesenchymal stem cells. *J Biomed Mater Res Part A* 101A:1651–1660
24. Thomas CE, Ehrhardt A, Kay MA (2003) Progress and problems with the use of viral vectors for gene therapy. *Nat Rev Genet* 4:346–358

25. Somia N, Verma IM (2000) Gene therapy: trials and tribulations. *Nat Rev Genet* 1:91–99
26. Verma IM, Weitzman MD (2005) Gene therapy: twenty-first century medicine. *Annu Rev Biochem* 74:711–738
27. Yang F, Green JJ, Dinio T, Keung L, Cho SW, Park H et al (2009) Gene delivery to human adult and embryonic cell-derived stem cells using biodegradable nanoparticulate polymeric vectors. *Gene Ther* 16:533–546
28. Evans CH (2012) Gene delivery to bone. *Adv Drug Deliv Rev* 64:1331–1340
29. Lundstrom K (2003) Latest development in viral vectors for gene therapy. *Trends Biotechnol* 21:117–122
30. Airene KJ, Hu Y-C, Kost TA, Smith RH, Kotin RM, Ono C et al (2013) Baculovirus: an insect-derived vector for diverse gene transfer applications. *Mol Ther* 21:739–749
31. Hu Y-C (2008) Baculoviral vectors for gene delivery: a review. *Curr Gene Ther* 8:54–65
32. Huang S, Kamihira M (2013) Development of hybrid viral vectors for gene therapy. *Biotechnol Adv* 31:208–223
33. Phillips JE, Gersbach CA, Garcia AJ (2007) Virus-based gene therapy strategies for bone regeneration. *Biomaterials* 28:211–229
34. Kofron MD, Laurencin CT (2006) Bone tissue engineering by gene delivery. *Adv Drug Deliv Rev* 58:555–576
35. Kimelman Bleich N, Kallai I, Lieberman JR, Schwarz EM, Pelled G, Gazit D (2012) Gene therapy approaches to regenerating bone. *Adv Drug Deliv Rev* 64:1320–1330
36. Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M et al (1995) T Lymphocyte-directed gene therapy for ADA – SCID: initial trial results after 4 years. *Science* 270:475–480
37. Hirschmann F, Verhoeven E, Wirth D, Bauwens S, Hauser H, Rudert M (2002) Vital marking of articular chondrocytes by retroviral infection using green fluorescence protein. *Osteoarthritis Cartilage* 10:109–118
38. Yao SY, Sukonnik T, Kean T, Bharadwaj RR, Pasceri P, Ellis J (2004) Retrovirus silencing, variegation, extinction and memory are controlled by a dynamic interplay of multiple epigenetic modifications. *Mol Ther* 10:27–36
39. Bandara G, Mueller GM, Galealauri J, Tindal MH, Georgescu HI, Suchanek MK et al (1993) Intraarticular expression of biologically active interleukin-1 receptor antagonist protein by ex vivo gene transfer. *Proc Natl Acad Sci U S A* 90:10764–10768
40. Cavazzana-Calvo M, Hacein-Bey S, Basile CD, Gross F, Yvon E, Nusbaum P et al (2000) Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 288:669–672
41. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P et al (2003) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 302:415–419
42. Fischer A, Cavazzana-Calvo M (2008) Gene therapy of inherited diseases. *Lancet* 371:2044–2047
43. Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E et al (2008) Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest* 118:3132–3142
44. Hacein-Bey-Abina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC et al (2010) Efficacy of gene therapy for X-linked severe combined immunodeficiency. *New Eng J Med* 363:355–364
45. Spitzer D, Hauser H, Wirth D (1999) Complement-protected amphotropic retroviruses from murine packaging cells. *Hum Gene Ther* 10:1893–1902
46. Gouze E, Pawliuk R, Pilpil C, Gouze JN, Fleet C, Palmer GD et al (2002) In vivo gene delivery to synovium by lentiviral vectors. *Mol Ther* 5:397–404
47. Li Y, Tew SR, Russell AM, Gonzalez KR, Hardingham TE, Hawkins RE (2004) Transduction of passaged human articular chondrocytes with adenoviral, retroviral, and lentiviral vectors and the effects of enhanced expression of SOX9. *Tissue Eng* 10:575–584
48. Yin GY, Liu WM, An P, Li P, Ding I, Planelles V et al (2002) Endostatin gene transfer inhibits joint angiogenesis and pannus formation in inflammatory arthritis. *Mol Ther* 5:547–554

49. Kato K, Miyake K, Igarashi T, Yoshino S, Shimada T (2005) Human immunodeficiency virus vector-mediated intra-articular expression of angiotensin II inhibits progression of collagen-induced arthritis in mice. *Rheumatol Int* 25:522–529
50. Cartier N, Hachein-Bey-Abina S, Bartholomae CC, Veres G, Schmidt M, Kutschera I et al (2009) Hematopoietic stem cell gene therapy with a lentiviral vector in X-Linked adrenoleukodystrophy. *Science* 326:818–823
51. Ronen K, Negre O, Roth S, Colomb C, Malani N, Denaro M et al (2011) Distribution of lentiviral vector integration sites in mice following therapeutic gene transfer to treat beta-thalassemia. *Mol Ther* 19:1273–1286
52. Heckl D, Schwarzer A, Haemmerle R, Steinemann D, Rudolph C, Skawran B et al (2012) Lentiviral vector induced insertional haploinsufficiency of Ebf1 causes murine leukemia. *Mol Ther* 20:1187–1195
53. Schambach A, Zychlinski D, Ehrnstroem B, Baum C (2013) Biosafety features of lentiviral vectors. *Hum Gene Ther* 24:132–142
54. Banasik MB, McCray PB (2009) Integrase-defective lentiviral vectors: progress and applications. *Gene Ther* 17:150–157
55. Chick HE, Nowrouzi A, Fronza R, McDonald RA, Kane NM, Alba R et al (2012) Integrase-deficient lentiviral vectors mediate efficient gene transfer to human vascular smooth muscle cells with minimal genotoxic risk. *Hum Gene Ther* 23:1247–1257
56. Imperiale MJ, Kochanek S (2004) Adenovirus vectors: biology, design, and production. *Curr Top Microbiol Immunol* 273:335–357
57. Roessler BJ, Allen ED, Wilson JM, Hartman JW, Davidson BL (1993) Adenoviral-mediated gene transfer to rabbit synovium in vivo. *J Clin Invest* 92:1085–1092
58. Yao QP, Wang SJ, Glorioso JC, Evans CH, Robbins PD, Ghivizzani SC et al (2001) Gene transfer of p53 to arthritic joints stimulates synovial apoptosis and inhibits inflammation. *Mol Ther* 3:901–910
59. Ghivizzani SC, Lechman ER, Kang R, Tio C, Kolls J, Evans CH et al (1998) 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 anti-arthritic effects. *Proc Natl Acad Sci U S A* 95:4613–4618
60. Brower-Toland BD, Saxer RA, Goodrich LR, Mi ZB, Robbins PD, Evans CH et al (2001) Direct adenovirus-mediated insulin-like growth factor I gene transfer enhances transplant chondrocyte function. *Hum Gene Ther* 12:117–129
61. Smith P, Shuler FD, Georgescu HI, Ghivizzani SC, Johnstone B, Niyibizi C et al (2000) Genetic enhancement of matrix synthesis by articular chondrocytes-Comparison of different growth factor genes in the presence and absence of interleukin-1. *Arthritis Rheum* 43:1156–1164
62. Nixon AJ, Haupt JL, Frisbie DD, Morisset SS, McIlwraith CW, Robbins PD et al (2005) Gene-mediated restoration of cartilage matrix by combination insulin-like factor-I/interleukin-1 receptor antagonist therapy. *Gene Ther* 12:177–186
63. Marshall E (1999) Gene therapy death prompts review of adenovirus vector. *Science* 286:2244–2245
64. Kochanek S, Clemens PR, Mitani K, Chen HH, Chan S, Caskey CT (1996) A new adenoviral vector: replacement of all viral coding sequences with 28 kb of DNA independently expressing both full-length dystrophin and beta-galactosidase. *Proc Natl Acad Sci U S A* 93:5731–5736
65. Evans CH, Gouze E, Gouze JN, Robbins PD, Ghivizzani SC (2006) Gene therapeutic approaches—transfer in vivo. *Adv Drug Deliv Rev* 58:243–258
66. Meager A (ed) (1999) Gene therapy technologies, applications and regulations. Wiley, New York
67. Mingozzi F, High KA (2011) Immune responses to AAV in clinical trials. *Curr Gene Ther* 11:321–330
68. Watanabe S, Imagawa T, Boivin GP, Gao GP, Wilson JM, Hirsch R (2000) Adeno-associated virus mediates long-term gene transfer and delivery of chondroprotective IL-4 to murine synovium. *Mol Ther* 2:147–152

69. Madry H, Cucchiari M, Terwilliger EF, Trippel SB (2003) Recombinant adeno-associated virus vectors efficiently and persistently transduce chondrocytes in normal and osteoarthritic human articular cartilage. *Hum Gene Ther* 14:393–402
70. Ulrich-Vinther M, Stengaard C, Schwarz EM, Goldring MB, Soballe K (2005) Adeno-associated vector mediated gene transfer of transforming growth factor- β 1 to normal and osteoarthritic human chondrocytes stimulates cartilage anabolism. *Arthritis Rheum* 46:2095–2104
71. Yokoo N, Saito T, Uesugi M, Kobayashi N, Xin K-Q, Okuda K et al (2005) Repair of articular cartilage defect by autologous transplantation of basic fibroblast growth factor gene-transduced chondrocytes with adeno-associated virus vector. *Arthritis Rheum* 52:164–170
72. Ito H, Koefoed M, Tiyyapattanaputi P, Gromov K, Goater JJ, Carmouche J et al (2005) Remodeling of cortical bone allografts mediated by adherent rAAV-RANKL and VEGF gene therapy. *Nat Med* 11:291–297
73. Yla-Herttuala S (2012) Endgame: glybera finally recommended for approval as the first gene therapy drug in the European Union. *Mol Ther* 20:1831–1832
74. Mease PJ, Wei N, Fudman EJ, Kivitz AJ, Schechtman J, Trapp RG et al (2010) 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* 37:692–703
75. Evans CH, Ghivizzani SC, Robbins PD (2008) Arthritis gene therapy's first death. *Arthritis Res Ther* 10:110
76. Frank KM, Hogarth DK, Miller JL, Mandal S, Mease PJ, Samulski RJ et al (2009) Brief report: investigation of the cause of death in a gene-therapy trial. *New Eng J Med* 361:161–169
77. Nayak S, Herzog RW (2010) Progress and prospects: immune responses to viral vectors. *Gene Ther* 17:295–304
78. McCarty DM (2008) Self-complementary AAV, vectors; advances and applications. *Mol Ther* 16:1648–1656
79. Smith RH, Levy JR, Kotin RM (2009) A simplified baculovirus-AAV expression vector system coupled with one-step affinity purification yields high-titer rAAV stocks from insect cells. *Mol Ther* 17:1888–1896
80. Kost TA, Condreay JP (1999) Recombinant baculoviruses as expression vectors for insect and mammalian cells. *Curr Opin Biotechnol* 10:428–433
81. Kost TA, Condreay JP (2002) Recombinant baculoviruses as mammalian cell gene delivery vectors. *Trends Biotechnol* 20:173–180
82. Hofmann C, Sandig V, Jennings G, Rudolph M, Schlag P, Strauss M (1995) Efficient gene-transfer into human hepatocytes by baculovirus vectors. *Proc Natl Acad Sci U S A* 92:10099–10103
83. Boyce FM, Bucher NLR (1996) Baculovirus-mediated gene transfer into mammalian cells. *Proc Natl Acad Sci U S A* 93:2348–2352
84. Kost TA, Condreay JP, Jarvis DL (2005) Baculovirus as versatile vectors for protein expression in insect and mammalian cells. *Nat Biotechnol* 23:567–575
85. Hu Y-C (2005) Baculovirus as a highly efficient expression vector in insect and mammalian cells. *Acta Pharmacol Sin* 26:405–416
86. Hu Y-C (2006) Baculovirus vectors for gene therapy. *Adv Virus Res* 68:287–320
87. Ho Y-C, Chen H-C, Wang K-C, Hu Y-C (2004) Highly efficient baculovirus-mediated gene transfer into rat chondrocytes. *Biotechnol Bioeng* 88:643–651
88. Wang K-C, Wu J-C, Chung Y-C, Ho Y-C, Chang MD, Hu Y-C (2005) Baculovirus as a highly efficient gene delivery vector for the expression of hepatitis delta virus antigens in mammalian cells. *Biotechnol Bioeng* 89:464–473
89. Merrihew RV, Clay WC, Condreay JP, Witherspoon SM, Dallas WS, Kost TA (2001) Chromosomal integration of transduced recombinant baculovirus DNA in mammalian cells. *J Virol* 75:903–909
90. Cheshenko N, Krougliak N, Eisensmith RC, Krougliak VA (2001) A novel system for the production of fully deleted adenovirus vectors that does not require helper adenovirus. *Gene Ther* 8:846–854

91. Jardin BA, Zhao Y, Selvaraj M, Montes J, Tran R, Prakash S et al (2008) Expression of SEAP (secreted alkaline phosphatase) by baculovirus mediated transduction of HEK 293 cells in a hollow fiber bioreactor system. *J Biotechnol* 135:272–280
92. Liu C-Y-Y, Chen H-Z, Chao Y-C (2010) Maximizing baculovirus-mediated foreign proteins expression in mammalian cells. *Curr Gene Ther* 10:232–241
93. Lesch HP, Laitinen A, Peixoto C, Vicente T, Makkonen KE, Laitinen L et al (2011) Production and purification of lentiviral vectors generated in 293T suspension cells with baculoviral vectors. *Gene Ther* 18:531–538
94. Lesch HP, Turpeinen S, Niskanen EA, Mähönen AJ, Airenne KJ, Ylä-Herttuala S (2008) Generation of lentivirus vectors using recombinant baculoviruses. *Gene Ther* 15:1280–1286
95. Huang K-S, Lo W-H, Chung Y-C, Lai Y-K, Chen C-Y, Chou S-T et al (2007) Combination of baculovirus-mediated gene delivery and packed-bed reactor for scalable production of adeno-associated virus. *Hum Gene Ther* 18:1161–1170
96. Matsuo E, Tani H, Lim C, Komoda Y, Okamoto T, Miyamoto H et al (2006) Characterization of HCV-like particles produced in a human hepatoma cell line by a recombinant baculovirus. *Biochem Biophys Res Commun* 340:200–208
97. Chen Y-H, Wu J-C, Wang K-C, Chiang Y-W, Lai C-W, Chung Y-C et al (2005) Baculovirus-mediated production of HDV-like particles in BHK cells using a novel oscillating bioreactor. *J Biotechnol* 118:135–147
98. Ernst W, Schinko T, Spenger A, Oker-Blom C, Grabherr R (2006) Improving baculovirus transduction of mammalian cells by surface display of a RGD-motif. *J Biotechnol* 126:237–240
99. Grabherr R, Ernst W (2010) Baculovirus for eukaryotic protein display. *Curr Gene Ther* 10:195–200
100. Hu Y-C, Yao K, Wu T-Y (2008) Baculovirus as an expression and/or delivery vehicle for vaccine antigens. *Expert Rev Vaccines* 7:363–371
101. Madhan S, Prabakaran M, Kwang J (2010) Baculovirus as vaccine vectors. *Curr Gene Ther* 10:201–213
102. Tani H, Abe T, Matsunaga TM, Miihi K, Matsuura Y (2008) Baculovirus vector for gene delivery and vaccine development. *Future Virol* 3:35–43
103. Wang S, Balasundaram G (2010) Potential cancer gene therapy by baculoviral transduction. *Curr Gene Ther* 10:214–225
104. Condeay JP, Ames RS, Hassan NJ, Kost TA, Merrihew RV, Mossakowska DE et al (2006) Baculoviruses and mammalian cell-based assays for drug screening. *Adv Virus Res* 68:255–286
105. Condeay JP, Kost TA (2007) Baculovirus expression vectors for insect and mammalian cells. *Curr Drug Targets* 8:1126–1131
106. Kost TA, Condeay JP, Ames RS (2010) Baculovirus gene delivery: a flexible assay development tool. *Curr Gene Ther* 10:168–173
107. Sung L-Y, Lo W-H, Chiu H-Y, Chen H-C, Chuang C-K, Lee H-P et al (2007) Modulation of chondrocyte phenotype via baculovirus-mediated growth factor expression. *Biomaterials* 28:3437–3447
108. Lo W-H, Hwang S-M, Chuang C-K, Chen C-Y, Hu Y-C (2009) Development of a hybrid baculoviral vector for sustained transgene expression. *Mol Ther* 17:658–666
109. Lu C-H, Lin K-J, Chiu H-Y, Chen C-Y, Yen T-C, Hwang S-M et al (2012) Improved chondrogenesis and engineered cartilage formation from TGF- β 3-expressing adipose-derived stem cells cultured in the rotating-shaft bioreactor. *Tissue Eng Part A* 18:2114–2124
110. Yeh T-S, Dean Fang Y-H, Lu C-H, Chiu S-C, Yeh C-L, Yen T-C et al (2014) Baculovirus-transduced, VEGF-expressing adipose-derived stem cell sheet for the treatment of myocardium infarction. *Biomaterials* 35:174–184
111. Ho Y-C, Lee H-P, Hwang S-M, Lo W-H, Chen H-C, Chung C-K et al (2006) Baculovirus transduction of human mesenchymal stem cell-derived progenitor cells: variation of transgene expression with cellular differentiation states. *Gene Ther* 13:1471–1479

112. Lin C-Y, Lu C-H, Luo W-Y, Chang Y-H, Sung L-Y, Chiu H-Y et al (2010) Baculovirus as a gene delivery vector for cartilage and bone tissue engineering. *Curr Gene Ther* 10:242–254
113. Wang C-Y, Wang S (2005) Adeno-associated virus inverted terminal repeats improve neuronal transgene expression mediated by baculoviral vectors in rat brain. *Hum Gene Ther* 16:1219–1226
114. Zeng J, Du J, Zhao Y, Palanisamy N, Wang S (2007) Baculoviral vector-mediated transient and stable transgene expression in human embryonic stem cells. *Stem Cells* 25:1055–1061
115. Chen C-L, Luo W-Y, Lo W-H, Lin K-J, Sung L-Y, Shih Y-S et al (2011) Development of hybrid baculovirus vectors for artificial MicroRNA delivery and prolonged gene suppression. *Biotechnol Bioeng* 108:2958–2967
116. Luo WY, Shih YS, Hung CL, Lo KW, Chiang CS, Lo WH et al (2012) Development of the hybrid Sleeping Beauty-baculovirus vector for sustained gene expression and cancer therapy. *Gene Ther* 19:844–851
117. Lin C-Y, Lin K-J, Kao C-Y, Chen M-C, Yen T-Z, Lo W-H et al (2011) The role of adipose-derived stem cells engineered with the persistently expressing hybrid baculovirus in the healing of massive bone defects. *Biomaterials* 32:6505–6514
118. Lu J, Zhang F, Kay MA (2013) A mini-intronic plasmid (MIP): a novel robust transgene expression vector in vivo and in vitro. *Mol Ther* 21:954–963
119. Gracey Maniar LE, Maniar JM, Chen Z-Y, Lu J, Fire AZ, Kay MA (2013) Minicircle DNA vectors achieve sustained expression reflected by active chromatin and transcriptional level. *Mol Ther* 21:131–138
120. Lin C-Y, Chang Y-H, Kao C-Y, Lu C-H, Sung L-Y, Yen T-C et al (2012) Augmented healing of critical-size calvarial defects by baculovirus-engineered MSCs that persistently express growth factors. *Biomaterials* 33:3682–3692
121. Raymond CS, Soriano P (2007) High-efficiency FLP and PhiC31 site-specific recombination in mammalian cells. *PLoS One* 2:e162
122. Turan S, Galla M, Ernst E, Qiao JH, Voelkel C, Schiedlmeier B et al (2011) Recombinase-mediated cassette exchange (RMCE): traditional concepts and current challenges. *J Mol Biol* 407:193–221
123. Sung LY, Chen CL, Lin SY, Hwang SM, Lu CH, Li KC et al (2013) Enhanced and prolonged baculovirus-mediated expression by incorporating recombinase system and in cis elements: a comparative study. *Nucleic Acids Res* 41:e139

Gene Therapy for Cartilage and Bone Tissue
Engineering

Hu, Y.-C.

2014, VIII, 89 p. 11 illus., 9 illus. in color., Softcover

ISBN: 978-3-642-53922-0