J Biosci (2019) 44:84
DOI: 10.1007/s12038-019-9905-3

Mini-Review

Biopolymers augment viral vectors based gene delivery

Balaji Balakrishnan[1] and Ernest David[2]*

[1]*Department of Haematology, Christian Medical College, Vellore 632004, Tamil Nadu, India*

[2]*Department of Biotechnology, Thiruvalluvar University, Vellore 632115, Tamil Nadu, India*

**Corresponding author (Email, ernestdavid2002@yahoo.com)*

MS received 24 January 2019; accepted 7 June 2019; published online 5 August 2019

The success of viral vectors mediated gene therapy is still hampered by immunogenicity and insufficient transgene expression. Alternatively, non-viral vectors mediated gene delivery has the advantage of low immunogenicity despite showing low transgene expression. By carefully considering the advantages of each approach, hybrid vectors are currently being developed by modifying the viral vectors using non-viral biopolymers. This review provides an overview of the hybrid vectors currently being developed.

Keywords. Biopolymers; gene delivery; gene therapy; vectors; virus

Gene delivery by viral vectors is by far the most successful method for gene therapy that has translated much from the clinical trial stage to gene therapy products in the market. Viruses like adenovirus, adeno-associated virus (AAV), retrovirus and lentivirus have been successfully explored in gene therapy (Lundstrom 2018). Viral vector-mediated gene therapy has shown great success in the treatment of lipoprotein lipase (LPL) deficiency (Miller 2012; Wierzbicki and Viljoen 2013), Leber's congenital amaurosis (Bainbridge *et al.* 2015), adenosine deaminase deficiency-severe combined immunodeficiency (ADA-SCID) (Aiuti *et al.* 2002) and hemophilia B (Nathwani *et al.* 2014; George *et al.* 2017). However this field has also seen pitfalls and limitations like withdrawal of Glybera, the gene therapy product to treat LPL deficiency (Senior 2017), risk of insertional mutagenesis in SCID (Howe *et al.* 2008), insufficient transgene expression (Bainbridge *et al.* 2015) and the immune response against vectors (Mingozzi and High 2013). Thus there is a constant need for improving the safety and efficacy of viral vector-based gene therapy. Systemic administration of viral vectors has always the risk of eliciting the host immune response which clears most viral particles thereby decreasing target tissue transduction efficacy. During systemic administration, the viral vectors face numerous barriers and so only a fraction of the viral vectors transduce the target tissues. Potential barriers could be: (i) the host immune system (complement system, pre-existing antibodies and capsid-specific immune response) and (ii) non-target cell types (like neutrophils, monocytes etc), endothelial cell layer present in the target tissue type. On the other hand, non-viral vectors like lipid-based or nanoparticle-based gene delivery (Midoux *et al.* 2009) exhibit transient, episomal expression of the transgene while having advantage of very low immunogenicity (figure 1).

By carefully considering the advantages of each approach, hybrid vectors are currently being developed by engineering these viral vectors to exhibit protective shields of non-viral vector materials like liposomes, nanoparticles or peptides (Waehler *et al.* 2007). Such hybrid vectors have several advantages like (i) evasion of the host immune response upon systemic administration; (ii) achieving optimum transgene expression efficacy at low-vector doses and (iii) a potential for targeted gene delivery upon incorporation of a targeting moiety. A great variety of materials have been used to coat/embed viral vectors to generate hybrid vectors. Here we present an updated overview of these hybrid vectors.

1. Liposomes

Encapsulation of retroviruses by liposomes was performed as early as 1984 when murine sarcoma viral vectors were encapsulated in unilamellar lipid vesicles prepared by a reverse-phase evaporation method by using cholesterol, phosphatidylcholine (PC) and phosphatidylglycerol. Such vectors improved the viral transduction efficiency remarkably (Faller and Baltimore 1984). Later in 1998, cationic liposome formulations were synthesized for retroviral encapsulation. Porter CD *et al.* encapsulated recombinant retroviruses in cationic lipids (a 3:2 (molar ratio) formulation

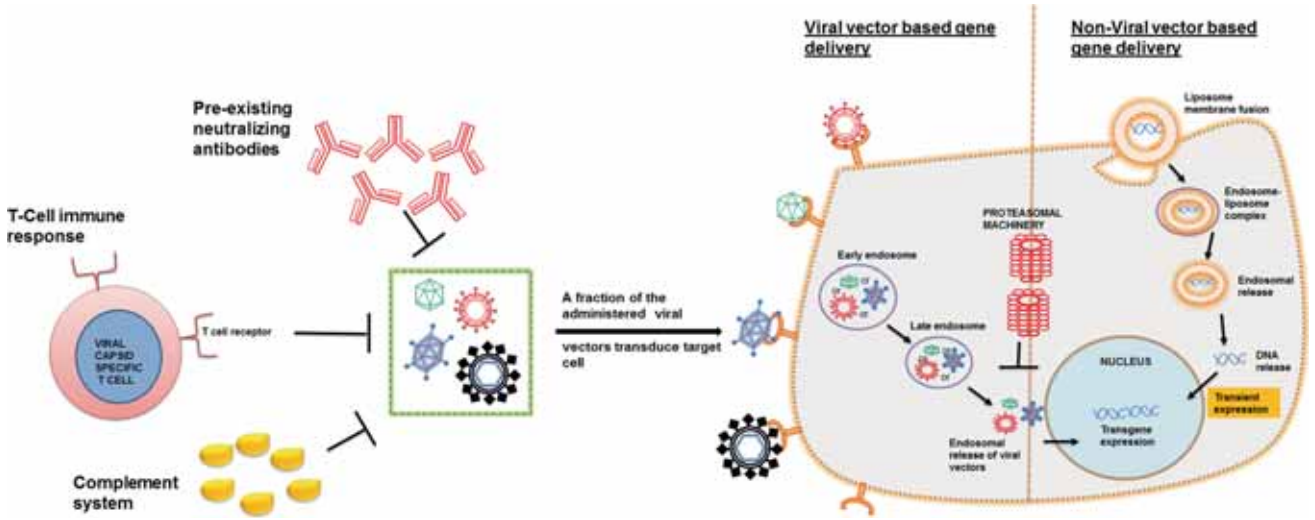

Figure 1. Schematic illustration of viral vectors and non-viral vector-mediated gene delivery. While viral vectors encounter barriers like the pre-existing neutralizing antibodies, complement system, capsid-specific immune response and proteasomal degradation of viral vectors, the non-viral vector-mediated transgene delivery is still hampered by insufficient and transient transgene expression.

of 3β[*N*-(*N*′,*N*′-dimethylaminoethane)carbamoyl]cholesterol (DC-Chol) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE)) and have found a 20-fold increase in transduction efficiency (Porter *et al.* 1998). Later, it was observed that high-cationic charge density can be reduced by including neutral lipids like DOPE and cholesterol thereby improving transfection efficiency. Such a neutral lipid/cationic lipid complex improves membrane fluidity, fuses with the endosomes and delivers the viral vectors inside the host cell efficiently (Crook *et al.* 1998). Keswani *et al.* produced a hybrid vector by combining murine leukemia virus-like particles (M-VLP) with synthetic liposomes comprising (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) (DOTAP), DOPE and cholesterol (φ/M-VLP). Such hybrid vectors were shown to have efficient size (300 nm produced by size extrusion of lipids), increased transfection efficiency and stable transgene expression for up to 3 weeks *in vitro* (Keswani *et al.* 2013).

Such a liposome encapsulation strategy has also been exploited with adenoviral vectors. Adenoviruses require the target cells to express receptors such as coxsackievirus and adenovirus receptor (CAR). However in cancer cells (CT-26 mouse colon cancer cells) which are deficient in CAR, adenoviruses complexed with PEGPE [1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(poly-ethyleneglycol)-2000] liposome showed enhanced transgene expression. Also in *in vivo* tumor models, it has been shown to be with reduced liver uptake and enhanced transgene expression in tumors (Han *et al.* 2008). Adenoviruses complexed with bilamellar DOTAP:chol liposomes have been reported to not only enhance transgene expression (LacZ or α_1-antitrypsin inhibitor (AAT)), but also was shown to evade anti-adenoviral neutralizing antibodies *in vivo* and *ex vivo* (Yotnda *et al.* 2002; Shi *et al.* 2013). Price *et al.* have also reported that adenovirus 5 encapsulated with dexamethasone-spermine (DS)/DOPE greatly enhanced transgene expression specifically in airway epithelium of C57Bl/6 mice when administrated through intranasal instillation. While they induced negligible expression in non-target alveolar cells, significantly evaded host IFNγ against the viruses (Price *et al.* 2005).

Cationic liposomes also have the disadvantages of decreased tissue targeting specificity (mediated by lipid fusion) and decreased serum stability (Ishida *et al.* 2002). Alternatively, adenovirus 5 have also been encapsulated in anionic liposomes comprising cholesterol derivatives, cholesteryl-hemisuccinate, egg PC and cholesterol which improved transgene expression 6-fold *in vivo* in airway epithelium (Zhong *et al.* 2011). This strategy was further refined by using non-toxic anionic lecithin–cholesterol–polyethylene glycol (PEG) liposomes to encapsulate adenoviral vectors which improved gene-transfer efficiency by 4-fold and safety as well (Mendez *et al.* 2014).

AAVs, the safe vector for gene delivery have also been encapsulated in liposomes comprising *N*-(α-trimethylammonio-acetyl)didodecyl-D-glutamate chloride, dilauroyl phosphatidylcholine and DOPE and have shown to transduce efficiently (>6-fold increase) in glioma cell lines which were otherwise difficult to transduce cell lines (Mizuno and Yoshida 1998). Furthermore, Fiandaca *et al.* have used this approach for imaging of AAV vectors *in vivo*. They encapsulated AAV1 with cationic lipids embedded with the MRI contrast agent, gadolinium and injected them in primate brain. This led to real-time MRI imaging visualization of convection-enhanced delivery of AAVs as well (Fiandaca *et al.* 2009). These cationic lipids were also observed to retarget AAV vectors. For example, AAV2/9 when encapsulated in cationic sterol-based lipid like DS, re-targeted from alveolar epithelium (native target) to airway epithelium with increased transduction efficiencies (up to 6-fold) both *in vitro* and *in vivo* thereby exhibiting potential for gene

transfer into lung for conditions like cystic fibrosis (Fein *et al.* 2009).

Herpes simplex viral (HSV) vectors employed mainly as oncolytic viral vectors are still not widely used due to their high immunogenicity. However as a proof-of-concept, Fu *et al.* employed this strategy and encapsulated HSV in DOTAP-based liposomes which was shown to evade host neutralization antibodies when administrated intravenously (Fu and Zhang 2001). Schematic representation of liposome-encapsulated viral vectors is illustrated in figure 2.

2. Modifying viral vectors using PEG

PEG is an amphiphilic and chemically inert polymer that has been widely used in food and pharmaceutical preparations due to the lack of toxicity and immunogenicity (Roberts *et al.* 2002). The process of modifying viral vectors by PEG is called as PEGylation (figure 3) and this has been reported to exhibit the following advantages:

(i) Increased transduction efficiency
(ii) Decreased immunogenicity
(iii) Improved stability

It is well known that adenoviruses upon systemic administration have a half-life of just 2 min where they get cleared by the host reticuloendothelial system (Alemany *et al.* 2000). PEG-modified adenoviruses had improved half-life by 4-fold compared to unmodified viruses and this increase in half-life further improves when the degree of PEGylation increases (Alemany *et al.* 2000; Wonganan and Croyle 2010). When adenoviruses were modified with 35 kDa PEG, the stability increases and resulted in viral genome being observed up to 24 h post systemic administration in mice (Hofherr *et al.* 2008). Further it was observed that PEGylation of adenoviruses greatly reduces hepatotoxicity and cytotoxicity as evidenced by measuring serum alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase while increasing transgene expression up to 28 days compared to unmodified virus (14 days) (Croyle *et al.* 2005; Wonganan *et al.* 2011). More importantly, modifying adenoviruses by monomethoxypolyethylene glycol activated by tresyl chloride (TMPEG) has also been reported to enhance transduction efficiency by more 2-fold *in vitro* in the presence of neutralizing antibodies and similar results were obtained *in vivo* in mice previously exposed to wild-type adenovirus vectors (O'Riordan *et al.* 1999). Such modification also improves the safety by greatly reducing serum IL6, IL12 and TNF-α (Croyle *et al.* 2005) and significantly reducing cytotoxic T lymphocytes (Croyle *et al.* 2001) and attenuating the Th2 response (Lanciotti *et al.* 2003).

AAVs when initially conjugated with monomethoxy poly(ethylene)glycols activated by tresyl chloride (TMPEG) and succinimidyl succinate (SSPEG), exhibited comparable transduction efficiencies with that of wild-type viruses while evading serum neutralization. However TMPEG also reduced the Th1-type response (Le *et al.* 2005; Lee *et al.* 2005). It is believed that covalent conjugation of PEG to reactive amino acids on the capsid might also occur at random sites thereby negatively affecting transduction potential. To avoid this, Yao *et al.* have performed site-specific PEGylation by incorporating azide moieties on selective regions such as Q325, S452 and R585 on AAV capsid followed by PEG conjugation. These vectors have been shown to exhibit 1.7 to 2.4 fold stability improvement in pooled human serum and a nearly two-fold reduction in antibody recognition (Yao *et al.* 2017).

Retroviruses modified with poly(ethylene glycol)-poly(L-lysine) block copolymer has been reported to increase transduction efficiency by 3–7 fold NIH3T3 or Lewis lung carcinoma cell lines. Interestingly this modification improved transduction efficiency in primary cultured brain cells which were otherwise labile (Katakura *et al.* 2004). It was also improvised by including a biotin moiety along with PEG (DSPE–PEG–biotin) while conjugating retroviruses and this strategy was shown to enhance virus binding to streptavidin by >3-fold (Mukherjee *et al.* 2009). Similarly VSV-G pseudotyped lentivirus vectors which are prone to inactivation by human serum complement, when conjugated with monomethoxypoly(ethylene)glycol, protected vector inactivation from complement while not affecting transduction efficiency *in vitro*. Such modification resulted in enhanced transgene expression in bone marrow and spleen up to 14 days post systemic administration (Croyle *et al.* 2004).

3. Magnetic nanoparticles

Another strategy to improve gene delivery is by conjugating supramagnetic nanoparticles (MNP) with the viral vectors. By means of magnetism principle, the vector–MNP conjugates are concentrated at the target site (figure 4). This technique is called as magnetofection (Plank *et al.* 2011). The main advantage of this method is to transduce difficult cell types owing to its receptor-independent endocytic uptake. Earliest example of such a modification is adenovirus-magnetic particles. When tested extensively in non-permissive COLO 205 cells, a human colon adenocarcinoma cell line with an applied magnetic field, enhanced transduction efficiency was observed (Pandori *et al.* 2002). Pereyra *et al.* have conjugated recombinant adenoviral vectors with iron oxide MNPs generating magneto-adenovectors (RAd-MNP). Such particles encoding GFP/insulin-like growth factor 1 were found to increase transduction efficiency in C2C12 myotubes *in vitro* and mouse muscles *in vivo* (Pereyra *et al.* 2016). Magnetofection has greatly improved using oncolytic adenoviruses guided them to tumors by magnetic field, a form of local delivery. When oncolytic adenoviruses dl520 were conjugated with iron oxide-based MPNs and tested *in vitro* in multidrug-resistant

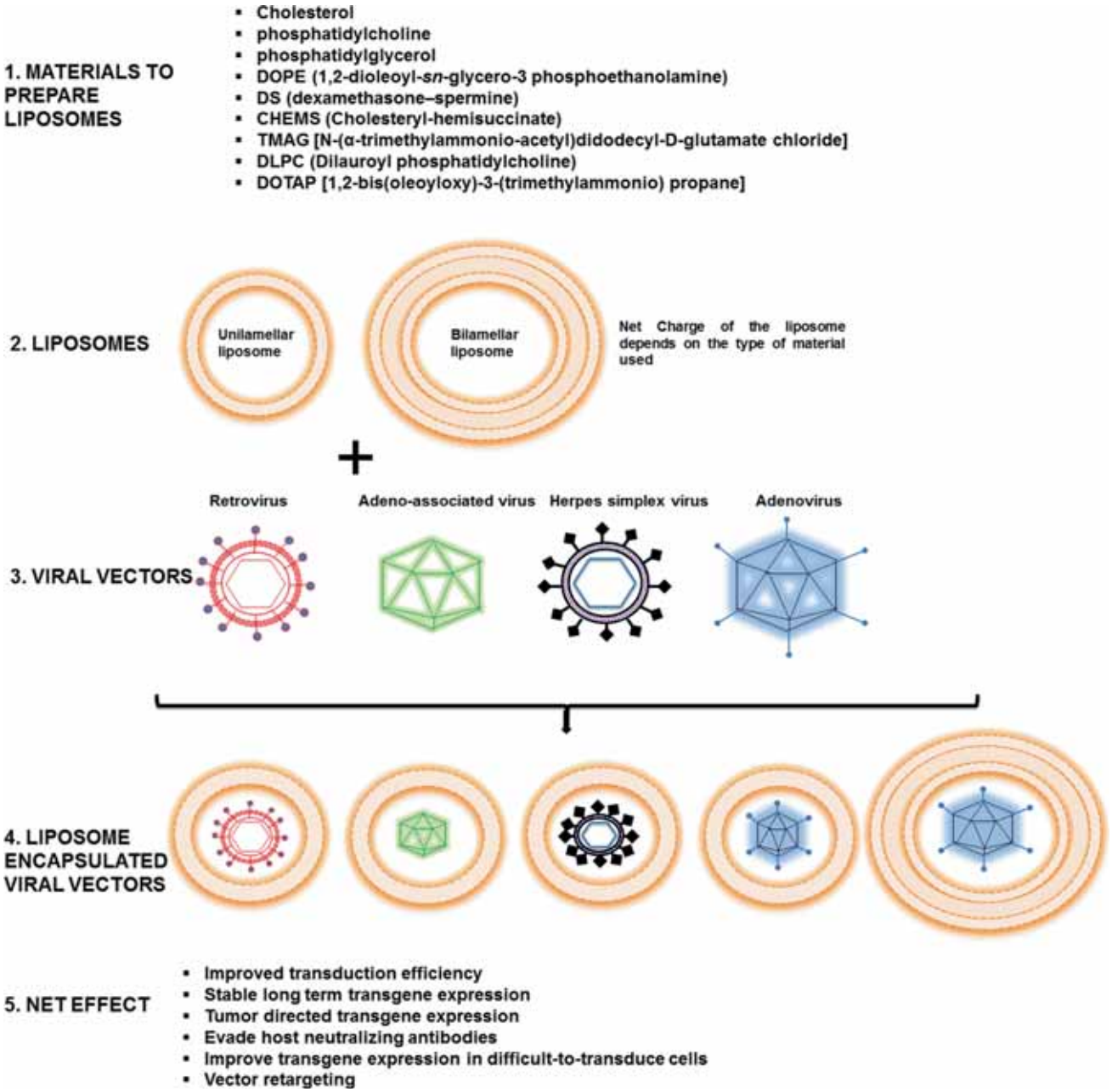

Figure 2. Liposomal encapsulation of viral vectors. Viral vectors can be encapsulated in liposomes made of materials as indicated in the figure that determines the net charge of the liposomes. Encapsulation can be unilamellar or bilamellar that help vectors improve transduction efficiency, evade pre-existing neutralization antibodies and also help in transducing cells that are otherwise difficult to transduce by viral vector alone.

cancer cells and *in vivo* by intratumoral injection followed by magnetic-field-guided transduction, 10-fold enhancement of the oncolytic potency was observed (Tresilwised *et al.* 2010). It was also observed that when adenoviruses were conjugated with gold/iron-oxide magnetic nanoparticles, transgene expression was improved 1000 times by direct penetration of the plasma membrane of mouse melanoma cells (Kamei *et al.* 2009).

In 2000, AAV2 were first coated with magnetic particles using a cleavable heparin sulfate linker. This AAV2-MPN was found to improve transduction efficiency in C12 cells *in vitro* and in 129/svJ mice *in vivo* upon intramuscular injection (Mah *et al.* 2002). Similarly, heparin-coated superparamagnetic nanoparticles were used to immobilize AAVr3.45 (which has diverse cell tropism). Such a system under magnetic field was found to enhance transduction efficiency *in vitro* in <180 min as against 24 h for the naked virus alone (Hwang *et al.* 2011). AAV2 is usually less permeable to neuronal cells. When AAV2 was genetically modified to display hexa-histidine (6xHis) on the capsid,

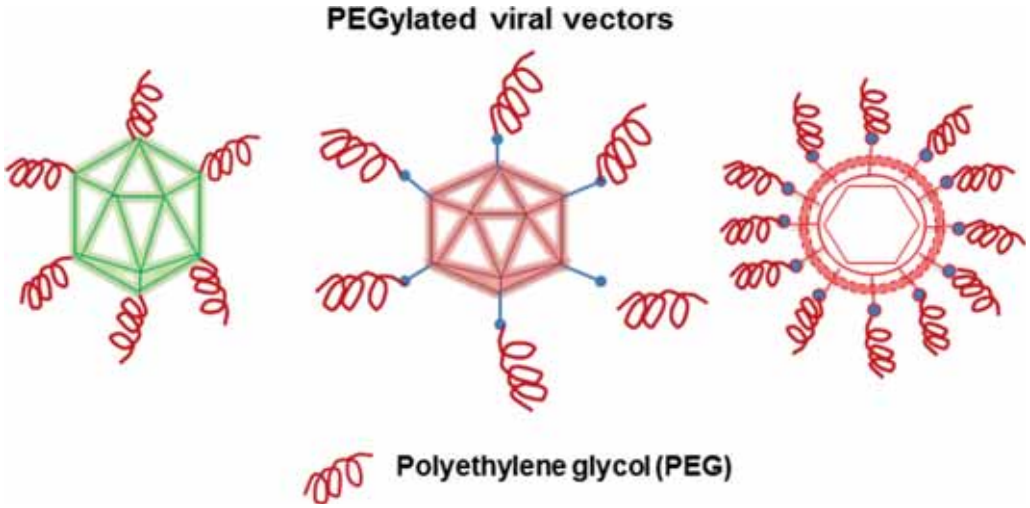

Figure 3. Schematic representation of PEGylation (addition of PEG moieties to the viral vectors).

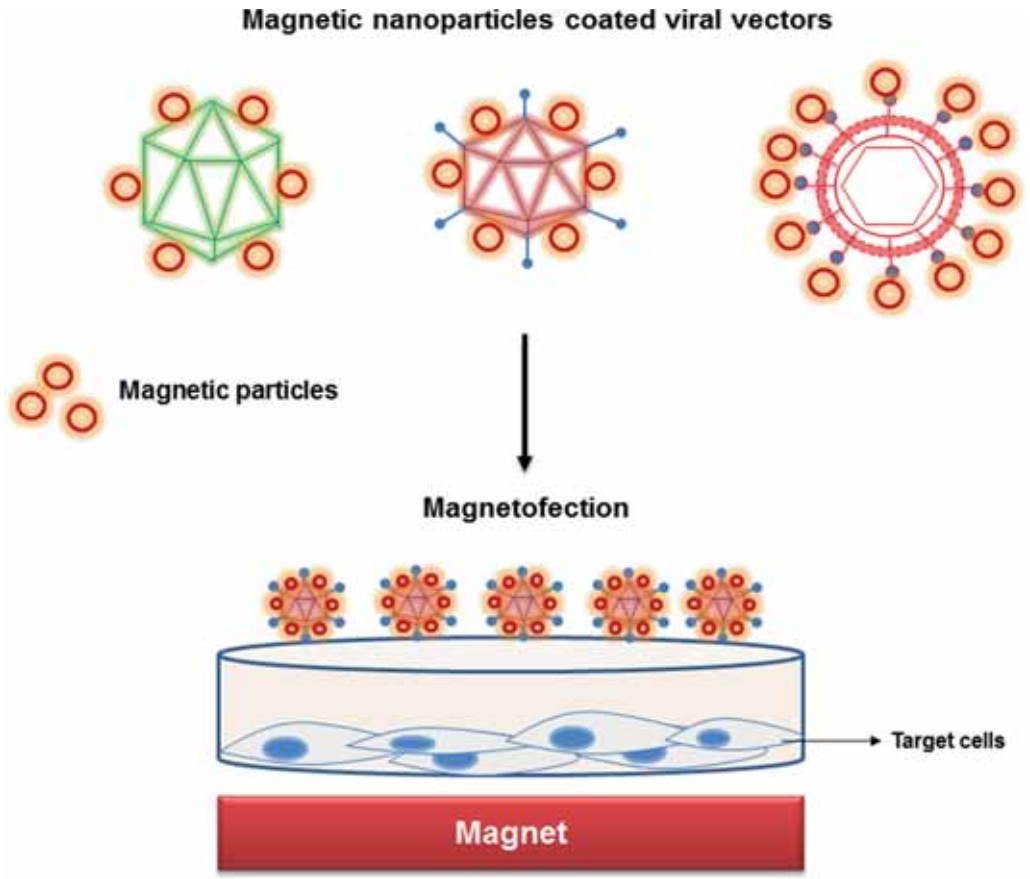

Figure 4. Magnetofection of viral particles. Magnetic nano-particles are complexed with the viral vectors. Based on the magnetism principle, the vector–magnetic nanoparticle complex was concentrated on the target cell types thereby increasing the transduction efficiency.

nickel ions – NTA-biotin conjugated to streptavidin-coated superparamagnetic iron oxide nanoparticles could be easily conjugated to develop AAV-MNPs. Under magnetic field guidance, enhanced transduction efficiency within 2–10 min of human neuronal stem cell infection was observed (Kim *et al.* 2011).

Retroviruses have also been complexed with poly-ethyleneimine-coated iron or gamma ferric oxide

nanoparticles and found to have intact physical and biological properties of the vector (Tai *et al.* 2003).

Hoffmann *et al.* have demonstrated that lentiviruses when conjugated to magnetic particles (CombiMag or TransMag) improved transduction efficiency in endothelial cells under hydrodynamic stress *in vitro* and observed the same in the *ex vivo* model of mouse aorta by the perfusion method guided by the magnetic field (Hofmann *et al.* 2009). Borroni *et al.* used this approach to enhance systemic and local delivery of lentiviruses. They complexed lentiviruses with iron oxide-based magnetic nanoparticles and administered intravenously in mice. Upon magnetic guidance to abdomen enhanced transduction in hepatocytes and non-parenchymous cells were observed. Similarly intra-tumoral delivery in a tumor model guided by the magnetic field enhanced transgene expression at the tumor, while preventing dissemination throughout the body (Borroni *et al.* 2017).

4. Conclusions

Targeted gene delivery using viral vectors in the field of gene therapy has reached great advances to date. However its success is still hampered by issues like immune-mediated clearance of viral particles and transient gene expression. Strategies to make these vectors evade the host immune response during systemic administration are of great value. By doing so, the amount of viral vectors needed for sufficient transgene expression will be low to moderate which in turn might reduce the cost associated with gene therapy. Thus strategies like modifying viral vectors using liposomes, PEG or magnetic nanoparticles give them the advantage of low immunogenicity and direct optimum transgene expression in the target tissue type.

References

Aiuti A, Slavin S, Aker M, Ficara F, Deola S, Mortellaro A, Morecki S, Andolfi G, Tabucchi A, Carlucci F, Marinello E, Cattaneo F, Vai S, Servida P, Miniero R, Roncarolo MG and Bordignon C 2002 Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. *Science* **296** 2410–2413

Alemany R, Suzuki K and Curiel DT 2000 Blood clearance rates of adenovirus type 5 in mice. *J. Gen. Virol.* **81** 2605–2609

Bainbridge JW, Mehat MS, Sundaram V, Robbie SJ, Barker SE, Ripamonti C, Georgiadis A, Mowat FM, Beattie SG, Gardner PJ, Feathers KL, Luong VA, Yzer S, Balaggan K, Viswanathan A, de Ravel TJ, Casteels I, Holder GE, Tyler N, Fitzke FW, Weleber RG, Nardini M, Moore AT, Thompson DA, Petersen-Jones SM, Michaelides M, van den Born LI, Stockman A, Smith AJ, Rubin G and Ali RR 2015 Long-term effect of gene therapy on Leber's congenital amaurosis. *N. Engl. J. Med.* **372** 1887–1897

Borroni E, Miola M, Ferraris S, Ricci G, Zuzek Rozman K, Kostevsek N, Catizone A, Rimondini L, Prat M, Verne E and Follenzi A 2017 Tumor targeting by lentiviral vectors combined with magnetic nanoparticles in mice. *Acta Biomater.* **59** 303–316

Crook K, Stevenson BJ, Dubouchet M and Porteous DJ 1998 Inclusion of cholesterol in DOTAP transfection complexes increases the delivery of DNA to cells in vitro in the presence of serum. *Gene Ther.* **5** 137–143

Croyle MA, Chirmule N, Zhang Y and Wilson JM 2001 'Stealth' adenoviruses blunt cell-mediated and humoral immune responses against the virus and allow for significant gene expression upon readministration in the lung. *J. Virol.* **75** 4792–4801

Croyle MA, Callahan SM, Auricchio A, Schumer G, Linse KD, Wilson JM, Brunner LJ and Kobinger GP 2004 PEGylation of a vesicular stomatitis virus G pseudotyped lentivirus vector prevents inactivation in serum. *J. Virol.* **78** 912–921

Croyle MA, Le HT, Linse KD, Cerullo V, Toietta G, Beaudet A and Pastore L 2005 PEGylated helper-dependent adenoviral vectors: Highly efficient vectors with an enhanced safety profile. *Gene Ther.* **12** 579–587

Faller DV and Baltimore D 1984 Liposome encapsulation of retrovirus allows efficient superinfection of resistant cell lines. *J. Virol.* **49** 269–272

Fein DE, Limberis MP, Maloney SF, Heath JM, Wilson JM and Diamond SL 2009 Cationic lipid formulations alter the *in vivo* tropism of AAV2/9 vector in lung. *Mol. Ther.: J. Am. Soc. Gene Ther.* **17** 2078–2087

Fiandaca MS, Varenika V, Eberling J, McKnight T, Bringas J, Pivirotto P, Beyer J, Hadaczek P, Bowers W, Park J, Federoff H, Forsayeth J and Bankiewicz KS 2009 Real-time MR imaging of adeno-associated viral vector delivery to the primate brain. *NeuroImage* **47** Suppl 2 T27–T35

Fu X and Zhang X 2001 Delivery of herpes simplex virus vectors through liposome formulation. *Mol. Ther.: J. Am. Soc. Gene Ther.* **4** 447–453

George LA, Sullivan SK, Giermasz A, Rasko JEJ, Samelson-Jones BJ, Ducore J, Cuker A, Sullivan LM, Majumdar S, Teitel J, McGuinn CE, Ragni MV, Luk AY, Hui D, Wright JF, Chen Y, Liu Y, Wachtel K, Winters A, Tiefenbacher S, Arruda VR, van der Loo JCM, Zelenaia O, Takefman D, Carr ME, Couto LB, Anguela XM and High KA 2017 Hemophilia B gene therapy with a high-specific-activity factor IX variant. *N. Engl. J. Med.* **377** 2215–2227

Han SY, Lee YJ, Jung HI, Lee SW, Lim SJ, Hong SH and Jeong JS 2008 Gene transfer using liposome-complexed adenovirus seems to overcome limitations due to coxsackievirus and adenovirus receptor-deficiency of cancer cells, both *in vitro* and *in vivo*. *Exp. Mol. Med.* **40** 427–434

Hofherr SE, Shashkova EV, Weaver EA, Khare R and Barry MA 2008 Modification of adenoviral vectors with polyethylene glycol modulates *in vivo* tissue tropism and gene expression. *Mol. Ther.: J. Am. Soc. Gene Ther.* **16** 1276–1282

Hofmann A, Wenzel D, Becher UM, Freitag DF, Klein AM, Eberbeck D, Schulte M, Zimmermann K, Bergemann C, Gleich B, Roell W, Weyh T, Trahms L, Nickenig G, Fleischmann BK and Pfeifer A 2009 Combined targeting of lentiviral vectors and positioning of transduced cells by magnetic nanoparticles. *Proc. Natl. Acad. Sci. U S A* **106** 44–49

Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kempski H, Brugman MH, Pike-Overzet K, Chatters SJ, de Ridder D, Gilmour KC, Adams S, Thornhill

SI, Parsley KL, Staal FJ, Gale RE, Linch DC, Bayford J, Brown L, Quaye M, Kinnon C, Ancliff P, Webb DK, Schmidt M, von Kalle C, Gaspar HB and Thrasher AJ 2008 Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J. Clin. Invest.* **118** 3143–3150

Hwang JH, Lee S, Kim E, Kim JS, Lee CH, Ahn IS and Jang JH 2011 Heparin-coated superparamagnetic nanoparticle-mediated adeno-associated virus delivery for enhancing cellular transduction. *Int. J. Pharm.* **421** 397–404

Ishida T, Harashima H and Kiwada H 2002 Liposome clearance. *Biosci. Rep.* **22** 197–224

Kamei K, Mukai Y, Kojima H, Yoshikawa T, Yoshikawa M, Kiyohara G, Yamamoto TA, Yoshioka Y, Okada N, Seino S and Nakagawa S 2009 Direct cell entry of gold/iron-oxide magnetic nanoparticles in adenovirus mediated gene delivery. *Biomaterials* **30** 1809–1814

Katakura H, Harada A, Kataoka K, Furusho M, Tanaka F, Wada H and Ikenaka K 2004 Improvement of retroviral vectors by coating with poly(ethylene glycol)-poly(L-lysine) block copolymer (PEG-PLL). *J. Gene. Med.* **6** 471–477

Keswani RK, Pozdol IM and Pack DW 2013 Design of hybrid lipid/retroviral-like particle gene delivery vectors. *Mol. Pharm.* **10** 1725–1735

Kim E, Oh JS, Ahn IS, Park KI and Jang JH 2011 Magnetically enhanced adeno-associated viral vector delivery for human neural stem cell infection. *Biomaterials* **32** 8654–8662

Lanciotti J, Song A, Doukas J, Sosnowski B, Pierce G, Gregory R, Wadsworth S and O'Riordan C 2003 Targeting adenoviral vectors using heterofunctional polyethylene glycol FGF2 conjugates. *Mol. Ther.: J. Am. Soc. Gene Ther.* **8** 99–107

Le HT, Yu QC, Wilson JM and Croyle MA 2005 Utility of PEGylated recombinant adeno-associated viruses for gene transfer. *J. Control. Release* **108** 161–177

Lee GK, Maheshri N, Kaspar B and Schaffer DV 2005 PEG conjugation moderately protects adeno-associated viral vectors against antibody neutralization. *Biotechnol. Bioeng.* **92** 24–34

Lundstrom K 2018 Viral vectors in gene therapy. *Diseases* **6** 1–20

Mah C, Fraites TJ Jr, Zolotukhin I, Song S, Flotte TR, Dobson J, Batich C and Byrne BJ 2002 Improved method of recombinant AAV2 delivery for systemic targeted gene therapy. *Mol. Ther.: J. Am. Soc. Gene Ther.* **6** 106–112

Mendez N, Herrera V, Zhang L, Hedjran F, Feuer R, Blair SL, Trogler WC, Reid TR and Kummel AC 2014 Encapsulation of adenovirus serotype 5 in anionic lecithin liposomes using a bead-based immunoprecipitation technique enhances transfection efficiency. *Biomaterials* **35** 9554–9561

Midoux P, Pichon C, Yaouanc JJ and Jaffres PA 2009 Chemical vectors for gene delivery: A current review on polymers, peptides and lipids containing histidine or imidazole as nucleic acids carriers. *Br. J. Pharmacol.* **157** 166–178

Miller N 2012 Glybera and the future of gene therapy in the European Union. *Nat. Rev. Drug Discovery* **11** 419

Mingozzi F and High KA 2013 Immune responses to AAV vectors: Overcoming barriers to successful gene therapy. *Blood* **122** 23–36

Mizuno M and Yoshida J 1998 Improvement of transduction efficiency of recombinant adeno-associated virus vector by entrapment in multilamellar liposomes. *Jpn. J. Cancer Res.* **89** 352–354

Mukherjee NG, Lyon LA and Le Doux JM 2009 Rapid modification of retroviruses using lipid conjugates. *Nanotechnology* **20** 065103

Nathwani AC, Reiss UM, Tuddenham EG, Rosales C, Chowdary P, McIntosh J, Della Peruta M, Lheriteau E, Patel N, Raj D, Riddell A, Pie J, Rangarajan S, Bevan D, Recht M, Shen YM, Halka KG, Basner-Tschakarjan E, Mingozzi F, High KA, Allay J, Kay MA, Ng CY, Zhou J, Cancio M, Morton CL, Gray JT, Srivastava D, Nienhuis AW and Davidoff A 2014 Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N. Engl. J. Med.* **371** 1994–2004

O'Riordan CR, Lachapelle A, Delgado C, Parkes V, Wadsworth SC, Smith AE and Francis GE 1999 PEGylation of adenovirus with retention of infectivity and protection from neutralizing antibody *in vitro* and *in vivo*. *Hum. Gene Ther.* **10** 1349–1358

Pandori M, Hobson D and Sano T 2002 Adenovirus-microbead conjugates possess enhanced infectivity: A new strategy for localized gene delivery. *Virology* **299** 204–212

Pereyra AS, Mykhaylyk O, Lockhart EF, Taylor JR, Delbono O, Goya RG, Plank C and Herenu CB 2016 Magnetofection enhances adenoviral vector-based gene delivery in skeletal muscle cells. *J. Nanomed. Nanotechnol.* **7** 1–11

Plank C, Zelphati O and Mykhaylyk O 2011 Magnetically enhanced nucleic acid delivery. Ten years of magnetofection-progress and prospects. *Adv. Drug Delivery Rev.* **63** 1300–1331

Porter CD, Lukacs KV, Box G, Takeuchi Y and Collins MK 1998 Cationic liposomes enhance the rate of transduction by a recombinant retroviral vector *in vitro* and *in vivo*. *J. Virol.* **72** 4832–4840

Price A, Limberis M, Gruneich JA, Wilson JM and Diamond SL 2005 Targeting viral-mediated transduction to the lung airway epithelium with the anti-inflammatory cationic lipid dexamethasone-spermine. *Mol. Ther.: J. Am. Soc. Gene Ther.* **12** 502–509

Roberts MJ, Bentley MD and Harris JM 2002 Chemistry for peptide and protein PEGylation. *Adv. Drug Delivery Rev.* **54** 459–476

Senior M 2017 After Glybera's withdrawal, what's next for gene therapy? *Nat. Biotechnol.* **35** 491–492

Shi HS, Yang LP, Wei W, Su XQ, Li XP, Li M, Luo ST, Zhang HL, Lu L, Mao YQ, Kan B and Yang L 2013 Systemically administered liposome-encapsulated Ad-PEDF potentiates the anti-cancer effects in mouse lung metastasis melanoma. *J. Transl. Med.* **11** 86

Tai M-F, Chi K-M, William Lau K-H, Baylink DJ and Chen S-T 2003 Generation of magnetic retroviral vectors with magnetic nanoparticles. *Rev. Adv. Mater. Sci.* **5** 5

Tresilwised N, Pithayanukul P, Mykhaylyk O, Holm PS, Holzmuller R, Anton M, Thalhammer S, Adiguzel D, Doblinger M and Plank C 2010 Boosting oncolytic adenovirus potency with magnetic nanoparticles and magnetic force. *Mol. Pharm.* **7** 1069–1089

Waehler R, Russell SJ and Curiel DT 2007 Engineering targeted viral vectors for gene therapy. *Nat. Rev. Genet.* **8** 573–587

Wierzbicki AS and Viljoen A 2013 Alipogene tiparvovec: Gene therapy for lipoprotein lipase deficiency. *Expert Opin. Biol. Ther.* **13** 7–10

Wonganan P and Croyle MA 2010 PEGylated adenoviruses: From mice to monkeys. *Viruses* **2** 468–502

Wonganan P, Clemens CC, Brasky K, Pastore L and Croyle MA 2011 Species differences in the pharmacology and toxicology of PEGylated helper-dependent adenovirus. *Mol. Pharm.* **8** 78–92

Yao T, Zhou X, Zhang C, Yu X, Tian Z, Zhang L and Zhou D 2017 Site-Specific PEGylated adeno-associated viruses with increased Serum stability and reduced immunogenicity. *Molecules* **22** pii: E1155

Yotnda P, Chen DH, Chiu W, Piedra PA, Davis A, Templeton NS and Brenner MK 2002 Bilamellar cationic liposomes protect adenovectors from preexisting humoral immune responses. *Mol. Ther.: J. Am. Soc. Gene Ther.* **5** 233–241

Zhong Z, Han J, Wan Y, Zhang Z and Sun X 2011 Anionic liposomes enhance and prolong adenovirus-mediated gene expression in airway epithelia *in vitro* and *in vivo*. *Mol. Pharm.* **8** 673–682

Corresponding editor: BJ Rao