Published in final edited form as:
Vision Res. 2008 February ; 48(3): 319–324.

Nanoparticle Applications in Ocular Gene Therapy

Xue Cai, **Shannon Conley**, and **Muna Naash***
Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Abstract

The use of nanoparticles as carriers for the delivery of therapeutic materials to target tissues has became popular in recent years and has demonstrated great potentials for the treatments of a wide range of diseases. In this review we summarize the advantages of nanotechnology as a common gene delivery strategy with emphasis on ocular therapy. Particular attention is paid to the CK30-PEG compacted DNA nanoparticles that have been successfully tested in the eye, lung, and brain. These particles resulted in higher transfection efficiency and longer duration of expression than other non-viral vectors without any toxicity or other side effects. They have been safely used clinically and are efficent for a broad range of gene therapy applications. The review also discusses mechanisms of nanoparticle uptake and internalization by cells, obstacles and limitations to the use of this technology, as well as novel methodologies to optimize nanoparticle driven gene expression.

Keywords

Nanoparticle; Ocular gene therapy; Gene transfer; Non; viral therapy; Retinal degeneration; Vector design

Utilizing nanoparticles for disease treatment in a wide range of medical research fields has become a popular strategy in recent years. These particles can serve as carriers for drugs, peptides, vaccines and oligonucleotides and have been successfully delivered to multiple targets including cancerous cells and other diseased tissues. Nanoparticles also have great potential as a strategy for gene therapy and can be used to treat genetic defects *in vitro* and *in vivo*.

Historically, viral vectors have been the preferred mechanism for transfer of nucleic acids into tissues of interest, and they have dominated the field for some time (Kay, Glorioso & Naldini, 2001). In a phase I/II clinical trial using a recombinant adeno-associated viral vector (rAAV) containing the herpes-simplex-virus thymidine kinase gene to treat hormone-refractory prostate cancer, two patients (out of six) responded positively to the therapy (Shirakawa, Gotoh, Terao et al., 2007).

The field of viral gene therapy in the retina has seen various successes. In a successful scenario, rAAV was used to transfer RPE65 cDNA to the RPE65-deficient eyes of Briard dogs. This treatment (and subsequent gene expression) led to the partial rescue of retinal function as demonstrated by electrophysiology and behavioral assessments. Furthermore, this restoration was stable for longer than three years without adverse effects (Acland, Aguirre, Bennett et al.,

* To whom correspondence should be addressed: Muna Naash, Department of Cell Biology, University of Oklahoma Health Sciences Center, 940 Stanton L. Young Blvd. BMSB 781, Oklahoma City, OK 73104, muna-naash@ouhsc.edu, 405-271-2388.

2005, Acland, Aguirre, Ray et al., 2001). Another example of a successful viral gene therapy treatment comes from a phase I clinical trial using AAV to deliver pigment epithelium-derived factor (PEDF) to the eyes of patients diagnosed with age-related macular degeneration (AMD) and showed a significant level of reduction in neoangiogenesis associated with disease progression (Campochiaro, Nguyen, Shah et al., 2006).

Interestingly, in spite of the fact that many retinal degenerative diseases are associated with a single mutation in phototransduction genes, there have been few positive reports for gene therapy-mediated rescue of such phenotypes. Modified HIV vectors have been used to preserve some retinal function in two recessive retinitis pigmentosa models: the Royal College of Surgeons rat (which carries a mutation in Mertk) and the *retinal degeneration* (*rd*) mouse (which carries a mutation in the beta subunit of cGMP phosphodiesterase) (Takahashi, Miyoshi, Verma et al., 1999, Tschernutter, Schlichtenbrede, Howe et al., 2005). Research on dominant retinal degenerations has focused mainly on ribozyme (Gorbatyuk, Justilien, Liu et al., 2007, Lewin, Drenser, Hauswirth et al., 1998) or RNAi mediated knockdown (Cashman, Binkley & Kumar-Singh, 2005) of the mutant proteins; such studies have reported moderate improvement on the disease phenotype. One of the exciting possibilities with RNAi involves non-mutation dependent knockdown, i.e. knockdown of all native protein with concurrent supplementation of a slightly modified wild-type protein that resists the RNAi treatment. In a proof-of-principle study designed to test the feasibility of this approach, researchers were able to specifically knockdown mouse rhodopsin expression (in cultured retinal explants) using short hairpin RNAs and concomitantly express the wild-type opsin (at ~90% of native levels) with silent mutations in the shRNA recognition sequence (Kiang, Palfi, Ader et al., 2005). It remains to be seen whether this strategy is applicable in an *in vivo* setting, but it represents an exciting approach to rescue diseases associated with gain-of-function mutations.

In spite of the use of improved viral vectors in these cases, rescue tends to be partial and of limited duration. Attempts to rescue retinal degenerations associated with structural proteins in the photoreceptors have had even less success. Ali's group reported that AAV-mediated delivery of peripherin 2 cDNA to the *retinal degeneration slow* (*Rds*$^{-/-}$) mutant mouse (null for peripherin 2/*Rds*) resulted in a transduction rate of only ~10% (Sarra, Stephens, de Alwis et al., 2001). Although the formation of outer segments was induced and moderate improvement in photoreceptor function was achieved, there was no significant attenuation of the severe photoreceptor cell loss seen in this model (Bainbridge, Tan & Ali, 2006, Sarra et al., 2001).

While these encouraging reports suggest that AAV-mediated treatments may be clinically useful, the viral approach still suffers from a number of physical limitations including random integration into the host's genome, immunogenicity of the vector, and limitations in the insert size (for rAAV, commonly reported as 4.7kB (Flotte, 2000)). More importantly, there can be significant toxic side effects such as stimulation of an immune response, inflammation and neutralizing antibodies associated with repeat treatment, and other potentially serious toxic outcomes including death (Davis & Cooper, 2007, Jackson, Juranek & Lipps, 2006, Thomas, Ehrhardt & Kay, 2003). Additionally, literature concerning the use of the most common AAV vectors for direct gene delivery is contradictory on both issues of transduction efficiency and inflammatory response and on the duration and reproducibility of transgene expression. The lack of a clearly superior viral candidate for future clinical application of gene therapy in the eye combined with the limitations of viral gene therapy mentioned above make the development of an efficacious non-viral vector for the eye of supreme importance.

There are several non-viral methods (Andrieu-Soler, Bejjani, de Bizemont et al., 2006) that have been sufficiently successful in delivering genes into many tissues and have potentials for clinical trials. These include liposomes, DNA nanoparticles (Davis & Cooper, 2007), or

combination of both (Hayes, Drummond, Kirpotin et al., 2006). Although liposomes are promising, they have shown low transfection efficiency and can cause significant inflammatory toxicity (Davis & Cooper, 2007). Alternatively, compacted DNA nanoparticles have proved to be a very useful vehicle for gene therapy and meet the majority of the requirements discussed above for a successful vector. Although there are many different formulations of nanoparticles, they typically contain a segment of DNA or RNA (circular or linear) which is compacted with a polycationic polymer. Their size is quite small with a typical range of 10–100 nm in diameter (Bondi, Azzolina, Craparo et al., 2007, Cooper, 2007, Davis & Cooper, 2007, Farjo, Skaggs, Quiambao et al., 2006, Fink, Klepcyk, Oette et al., 2006, Hayes et al., 2006, Konstan, Davis, Wagener et al., 2004, Lee, Zhang, Shirley et al., 2007, Liu, Li, Pasumarthy et al., 2003, Zhang, Zhang, Chen et al., 2007b, Ziady, Gedeon, Miller et al., 2003). These small particles are taken up at the cell surface and trafficked to the nucleus within a short period of time. Delivery of compacted DNA nanoparticles to the target yields medium to high transfection efficiency; in many cases expression levels are several folds greater than those observed after treatment with naked plasmid DNA. In one instance the expression level was 200-fold higher than treatment with the naked plasmid (Ziady et al., 2003). These results are dependent on specifics of the nanoparticle formulation, size, or electric charge (Bondi et al., 2007, Cooper, 2007, Davis & Cooper, 2007, Fink et al., 2006, Harush-Frenkel, Debotton, Benita et al., 2007, Liu et al., 2003, Walsh, Tangney, O'Neill et al., 2006). Excellent preliminary studies have been undertaken with polylactic acid and polylactide co-glycolide nanoparticles in the retina, but so far they have not been used for gene transfer to the mammalian retina (Bejjani, BenEzra, Cohen et al., 2005, Bourges, Gautier, Delie et al., 2003). Compacted polyethylene glycol (PEG) nanoparticles have been used to efficiently transfect post-mitotic cells *in vitro* and *in vivo* (Cooper, 2007, Davis & Cooper, 2007, Farjo et al., 2006, Fink et al., 2006, Lee et al., 2007, Liu et al., 2003, Yurek, Fletcher-Turner & Cooper, 2005, Ziady et al., 2003). Additionally, these nanoparticles can be stably stored under a variety of conditions and concentrations (up to 12 mg/ml of DNA); they are tolerant of a wide range of temperatures, salt concentrations and pH; and they tend to protect their DNA or RNA from DNase or RNase degradation (Bondi et al., 2007, Cooper, 2007, Davis & Cooper, 2007, Farjo et al., 2006, Fink et al., 2006, Guo, 2005, Hayes et al., 2006, Lee et al., 2007, Liu et al., 2003, Sesenoglu-Laird, Svenson, Tyr et al., 2007). One of the most exciting features of compacted DNA/RNA nanoparticles is their insert capacity; some DNA-compacted nanoparticles can contain plasmids up to 20 kb and retain full functional competence following *in vivo* administration (Fink et al., 2006). Studies in humans and mice showed little to no toxicity in the targeted tissues, and modest immune response when high concentration of the nanoparticles is used (Cooper, 2007, Farjo et al., 2006, Konstan et al., 2004). The lack of serious side effects after treatment indicates that repetitive administration of the nanoparticles is possible which adds another advantage over some viral vectors (Bourges et al., 2003, Cooper, 2007, Davis & Cooper, 2007). Recently, nanoparticles have had some success in phase I/II clinical trials designed to treat cystic fibrosis. The nanoparticles used for those studies were compacted with a lysine 30-mer linked to 10 kDa polyethylene glycol (PEG) and contained CMV-CFTR cDNA. The success of this trial highlights the clinical utility of this new technology as an effective gene delivery vector *in vivo* (Konstan et al., 2004). DNA nanoparticles can also be used to deliver RNA (for RNA interference) to the diseased tissues to help treat dominant genetic diseases. RNA nanoparticles have been used to suppress malignant growth by inducing apoptosis in human lung cancer cells (Guo, 2005, Li & Huang, 2006). In spite of these successes, there are still barriers to the universal application of this technology for the treatment of human diseases. The biggest problem so far has been the low transfection efficiency seen with some particles and the short duration of gene expression which is typically associated with most non-viral gene therapies.

In an effort to develop such a non-viral strategy, our lab has been cooperating with Copernicus Therapeutics, Inc., to optimize an exciting type of compacted-DNA nanoparticle for use in the treatment of genetic retinal degenerations. These nanoparticles are comprised of 30-mer lysine

polymers substituted with 10 kDa PEG and can be used to compact any type of nucleic acid. One of the unique features of these particles that contribute to their very small size (8–20 nm in diameter) is that each particle contains only one molecule of DNA. Various shapes of nanoparticles can be achieved by varying the polylysine counterion present at the time of compaction (Cooper, 2007, Fink et al., 2006, Guo, 2005, Liu et al., 2003). This formulation option helps facilitate the development of customized nanoparticles for use in different cell types (Farjo et al., 2006, Fink et al., 2006, Kowalczyk, Pasumarthy, Gedeon et al., 2001, Liu et al., 2003, Ziady et al., 2003). The particles are rod-like or ellipsoidal in shape when compacted in the presence of either acetate (AC) or trifluoroacetate (TFA) counterions, respectively. The AC (or TFA)-CK30-PEG DNA nanoparticles have been demonstrated to be non-immunogenic, non-inflammatory, and non-toxic in various tissues. They robustly transfect various non-dividing tissues *in vivo* with efficiencies comparable to and up to 10-fold higher than any viral vector: ~30%–80% of lung cells and >95% of retinal cells were transfected. Furthermore, transfection efficiency in brain tissues was comparable to those seen with viral vectors (Cooper, 2007).

We have delivered these nanoparticles (containing CMV-EGFP DNA and compacted with either AC or TFA) to ocular tissues of adult wild type mice by intravitreal or subretinal injection (Farjo et al., 2006). Two days post injection, the injected eyes were harvested and GFP expression levels were analyzed by qRT-PCR and immunohistochemistry. The results demonstrated that these nanoparticles can be targeted to different tissues within the eye by varying the injection site. Almost all cell types of the eye were transfected by the nanoparticles and demonstrated robust and dose-dependent levels of transgene expression. Intravitreal injections drove strong GFP expression in the inner retina (in the ganglion cell and inner plexiform layers) and in other more anterior ocular tissues, e.g. in cornea, trabecular meshwork, and lens. Conversely, subretinal injection drove strong GFP expression in the outer nuclear layer and retinal pigment epithelium as well as in the optic nerve of the eyes. Nanoparticle uptake was not limited to the site of injection and there was no evidence of cellular infiltration or inflammation. These data strongly suggest that compacted-DNA nanoparticles are suitable for application to multiple types of ocular diseases. Since intravitreal injection targets the tissue in the front of the eye, this mode of therapy could be widely used for corneal disease treatment while subretinal injection could be used for diseases of the optic nerve and the retina. This system provides tremendous promise for developing gene therapy strategies to treat various ocular diseases (Farjo et al., 2006).

One of the traditional limitations of non-viral vectors has been passage of the vector across two physiological barriers: the cell membrane and the nuclear membrane. The most commonly accepted pathway for nanoparticle internalization into the cytosol is endocytosis (Walsh et al., 2006, Zhang, Chen, Zhang et al., 2007a). Endocytosis can be divided into two categories, phagocytosis (which requires specialized cells) or pinocytosis. The latter pathway can be subdivided into macropinocytosis (molecules >120 nm), caveolin-mediated endocytosis (molecules ~60 nm) and clathrin-mediated endocytosis (~120 nm) (Conner & Schmid, 2003). Positively charged nanoparticles are known to use the clathrin-mediated endocytosis (Harush-Frenkel et al., 2007). It was shown that C1K30-PEG (a similar formulation to that used by Liu et al 2003) did not colocalize with markers of clathrin-coated endosomes (transferrin receptor), or late endosomes/lysosomes (lysobisphosphatidic acid) (Walsh et al., 2006). Furthermore, nanoparticle entry into the cell was not blocked by the presence of the inhibitors of clathrin or caveolae-mediated uptake, chlorpromazine and filipin III, respectively, but was inhibited by amiloride (an inhibitor of the Na^+/H^+ exchange required for macropinocytosis) suggesting that C1K30-PEG nanoparticles enter cells via a different pathway than previously described for poly-L-lysine compacted vectors (Walsh et al., 2006). The high transfection efficiency we (and others) have observed with these nanoparticles suggests that they are adept at crossing the nuclear membrane. It has been reported that the CK_{30}PEG10k nanoparticles are less than 24–

25 nm in diameter which would permit efficient nuclear uptake across the 25 nm nuclear membrane pores (Davis & Cooper, 2007, Liu et al., 2003). This hypothesis is supported by the observation that transfection was inhibited in the presence of wheat germ agglutinin (WGA), which blocks the nuclear membrane pore (Davis & Cooper, 2007, Liu et al., 2003). However, the observation that even particles bigger than 25nm have shown efficient nuclear uptake which led investigators to further study nanoparticle trafficking. To identify the exact uptake mechanism used by our compacted-DNA nanoparticles, the plasmid DNA was labeled with rhodamine prior to compaction and tracked the particles over time (Chen & Davis, 2007). Based on immunolocalization and biochemical studies, these nanoparticles colocalized with a nuclear/plasma membrane protein called nucleolin. Cotransport of the nanoparticles and nucleolin was traced at different time points in Hela cells; after the nanoparticles were transported through the cell membrane into the cytoplasm they were then trafficked to the nucleus by a nucleolin-mediated pathway (Chen & Davis, 2006, Chen & Davis, 2007, Cooper, 2007). Cellular uptake and nuclear localization is independent of the DNA plasmid payload and gene transfer in cells can be efficiently blocked by excess purified nucleolin protein added to the media (Chen & Davis, 2006). DNA nanoparticles bind to nucleolin with nanomolar affinities (Chen & Davis, 2006). These results clearly showed that cells that express nucleolin on the cell surface can take up the DNA nanoparticles. Furthermore, it is possible to retarget these DNA nanoparticles to other tissues, such as liver, by adding cell-specific ligands to the nanoparticles (Sun, Rosenblum, Lee et al., 2007).

An additional limitation of non-viral vectors has been short duration of expression. Although our compacted-DNA nanoparticles exhibit high transfection efficiency, initial studies employing the CMV immediate-early promoter indicated that expression was temporary (two weeks) (Farjo et al., 2006). This short duration was likely due to silencing of the CMV promoter (Bauer, Maier, Neyses et al., 2005), (Chen, He, Meuse et al., 2004), and attempts to maximize duration of gene expression are critical. In order to achieve long term tissue-specific transgene expression *in vivo*, many modifications to the nanoparticles have been performed. These include surface modification of the nanoparticles with various coatings and targeting ligands (Li, Yu, Huang et al., 2007, Li & Huang, 2006, Zhang et al., 2007b) and the use of tissue specific or strong promoters as well as a variety of prokaryotic backbone elements. Recently, an idea was proposed suggesting the incorporation of some features of native chromosomes into the design of extrachromosomal gene expression vectors can improve transgene expression. These modifications are based on the knowledge of the genome of the animal species in question, with particular reference to the histone code, transcription factors and the transcriptional machinery, nuclear organization, and structure of the nucleoskeleton and nuclear matrix (Jackson et al., 2006). When a plasmid containing a scaffold/matrix attachment region (S/MAR) element combined with the alpha-1 antitrypsin promoter (AAT) and the luciferase gene was hydrodynamically delivered to the mouse liver, 70% of cells were transfected and gene expression was sustained for 6 months (a significant improvement over traditional plasmids) (Jackson et al., 2006). This S/MAR sequence also protects the promoter from methylation and subsequent silencing of the transgene (Argyros, Wong, Waddingotn et al., 2007).

An alternate approach to increasing the duration of gene expression was taken by Chen et al. who reported that minicircle plasmids in which all bacterial sequences were eliminated (including the bacterial origin of replication and the antibiotic resistance gene) promoted gene expression that persisted for several years in the liver (Chen, He, Ehrhardt et al., 2003, Chen, He & Kay, 2005). Our group is working on implementing some of these exciting ideas in conjunction with the compacted DNA nanoparticles to optimize gene expression in the eye. We are utilizing linearized DNAs, minicircle DNAs, and plasmids containing S/MAR regions in our nanoparticles in an attempt to increase the duration and levels of transgene expression. Our group's most recent studies have used compacted DNA nanoparticles to transfer the normal

mouse peripherin 2 (NMP), a product of the RDS gene, under the control of tissue specific promoters to the *Rds* heterozygous retina, a mouse model of autosomal dominant retinitis pigmentosa (ADRP). We have demonstrated that transfection efficiency is above 90% and have achieved partial structural and functional rescue (Cai, Fliesler, Cooper et al., 2007). Interestingly, both in this case and in some other studies done in the lung, transgene expression was sustained for several months suggesting that additional vector modifications may not be necessary in all cases (Cai et al., 2007).

In summary, compacted-DNA nanoparticle mediated gene therapy provides a safe, effective and promising system for the delivery of therapeutic genes to target tissues in the eye. They drive very specific and high levels of gene expression and the expression can be sustained for several months. The safe use of compacted DNA nanoparticles in the clinical setting speaks to their viability as a potential treatment strategy for human conditions (Konstan et al., 2004). The use of this system in the treatment of genetic diseases of the eye promises to be a strong alternative to the existing collection of viral vectors.

References

Acland GM, Aguirre GD, Bennett J, Aleman TS, Cideciyan AV, Bennicelli J, Dejneka NS, Pearce-Kelling SE, Maguire AM, Palczewski K, Hauswirth WW, Jacobson SG. Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. Mol Ther 2005;12:1072–1082. [PubMed: 16226919]

Acland GM, Aguirre GD, Ray J, Zhang Q, Aleman TS, Cideciyan AV, Pearce-Kelling SE, Anand V, Zeng Y, Maguire AM, Jacobson SG, Hauswirth WW, Bennett J. Gene therapy restores vision in a canine model of childhood blindness. Nat Genet 2001;28:92–95. [PubMed: 11326284]

Andrieu-Soler C, Bejjani RA, de Bizemont T, Normand N, BenEzra D, Behar-Cohen F. Ocular gene therapy: a review of nonviral strategies. Mol Vis 2006;12:1334–1347. [PubMed: 17110916]

Argyros O, Wong SP, Waddingotn S, Coutelle C, Miller A, Harbottle RP. Development of S-MAR plasmid vectors for persistent expression and maintenance, *in vivo*. Molecular Therapy 2007;15:S130.

Bainbridge JW, Tan MH, Ali RR. Gene therapy progress and prospects: the eye. Gene Ther 2006;13:1191–1197. [PubMed: 16838031]

Bauer S, Maier SK, Neyses L, Maass AH. Optimization of gene transfer into neonatal rat cardiomyocytes and unmasking of cytomegalovirus promoter silencing. DNA Cell Biol 2005;24:381–387. [PubMed: 15941390]

Bejjani RA, BenEzra D, Cohen H, Rieger J, Andrieu C, Jeanny JC, Gollomb G, Behar-Cohen FF. Nanoparticles for gene delivery to retinal pigment epithelial cells. Mol Vis 2005;11:124–132. [PubMed: 15735602]

Bondi ML, Azzolina A, Craparo EF, Lampiasi N, Capuano G, Giammona G, Cervello M. Novel cationic solid-lipid nanoparticles as non-viral vectors for gene delivery. J Drug Target 2007;15:295–301. [PubMed: 17487698]

Bourges JL, Gautier SE, Delie F, Bejjani RA, Jeanny JC, Gurny R, BenEzra D, Behar-Cohen FF. Ocular drug delivery targeting the retina and retinal pigment epithelium using polylactide nanoparticles. Invest Ophthalmol Vis Sci 2003;44:3562–3569. [PubMed: 12882808]

Cai X, Fliesler SJ, Cooper M, Naash MI. Functional rescue of the Rds haploinsufficency phonotype with non-viral compacted DNA nanoparticles. Molecular Therapy 2007;15:S347.

Campochiaro PA, Nguyen QD, Shah SM, Klein ML, Holz E, Frank RN, Saperstein DA, Gupta A, Stout JT, Macko J, DiBartolomeo R, Wei LL. Adenoviral vector-delivered pigment epithelium-derived factor for neovascular age-related macular degeneration: results of a phase I clinical trial. Hum Gene Ther 2006;17:167–176. [PubMed: 16454650]

Cashman SM, Binkley EA, Kumar-Singh R. Towards mutation-independent silencing of genes involved in retinal degeneration by RNA interference. Gene Ther 2005;12:1223–1228. [PubMed: 15877050]

Chen X, Davis P. Compacted DNA nanoparticles transfect cells by binding to cell surface nucleolin. Molecular Therapy 2006;13:S152.

Chen X, Davis P. Intracellular trafficking of DNA nanoparticle and nucleolin antibody. Molecular Therapy 2007;15:S196.

Chen ZY, He CY, Ehrhardt A, Kay MA. Minicircle DNA vectors devoid of bacterial DNA result in persistent and high-level transgene expression in vivo. Mol Ther 2003;8:495–500. [PubMed: 12946323]

Chen ZY, He CY, Kay MA. Improved production and purification of minicircle DNA vector free of plasmid bacterial sequences and capable of persistent transgene expression in vivo. Hum Gene Ther 2005;16:126–131. [PubMed: 15703495]

Chen ZY, He CY, Meuse L, Kay MA. Silencing of episomal transgene expression by plasmid bacterial DNA elements in vivo. Gene Ther 2004;11:856–864. [PubMed: 15029228]

Conner SD, Schmid SL. Regulated portals of entry into the cell. Nature 2003;422:37–44. [PubMed: 12621426]

Cooper, MJ. Nonviral gene transfer in the lung: from animal to human. Molecular therapy, S15, Vii Scientific symposium 315: Non-viral gene therapy: toward nonviral gene therapy; 2007.

Davis PB, Cooper MJ. Vectors for airway gene delivery. Aaps J 2007;9:E11–17. [PubMed: 17408235]

Farjo R, Skaggs J, Quiambao AB, Cooper MJ, Naash MI. Efficient non-viral ocular gene transfer with compacted DNA nanoparticles. PLoS ONE 2006;1:e38. [PubMed: 17183666]

Fink TL, Klepcyk PJ, Oette SM, Gedeon CR, Hyatt SL, Kowalczyk TH, Moen RC, Cooper MJ. Plasmid size up to 20 kbp does not limit effective in vivo lung gene transfer using compacted DNA nanoparticles. Gene Ther 2006;13:1048–1051. [PubMed: 16525478]

Flotte TR. Size does matter: overcoming the adeno-associated virus packaging limit. Respir Res 2000;1:16–18. [PubMed: 11667959]

Gorbatyuk M, Justilien V, Liu J, Hauswirth WW, Lewin AS. Preservation of photoreceptor morphology and function in P23H rats using an allele independent ribozyme. Exp Eye Res 2007;84:44–52. [PubMed: 17083931]

Guo P. RNA nanotechnology: engineering, assembly and applications in detection, gene delivery and therapy. J Nanosci Nanotechnol 2005;5:1964–1982. [PubMed: 16430131]

Harush-Frenkel O, Debotton N, Benita S, Altschuler Y. Targeting of nanoparticles to the clathrin-mediated endocytic pathway. Biochem Biophys Res Commun 2007;353:26–32. [PubMed: 17184736]

Hayes ME, Drummond DC, Kirpotin DB, Zheng WW, Noble CO, Park JW, Marks JD, Benz CC, Hong K. Genospheres: self-assembling nucleic acid-lipid nanoparticles suitable for targeted gene delivery. Gene Ther 2006;13:646–651. [PubMed: 16341056]

Jackson DA, Juranek S, Lipps HJ. Designing nonviral vectors for efficient gene transfer and long-term gene expression. Mol Ther 2006;14:613–626. [PubMed: 16784894]

Kay MA, Glorioso JC, Naldini L. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. Nat Med 2001;7:33–40. [PubMed: 11135613]

Kiang AS, Palfi A, Ader M, Kenna PF, Millington-Ward S, Clark G, Kennan A, O'Reilly M, Tam LC, Aherne A, McNally N, Humphries P, Farrar GJ. Toward a gene therapy for dominant disease: validation of an RNA interference-based mutation-independent approach. Mol Ther 2005;12:555–561. [PubMed: 15967729]

Konstan MW, Davis PB, Wagener JS, Hilliard KA, Stern RC, Milgram LJ, Kowalczyk TH, Hyatt SL, Fink TL, Gedeon CR, Oette SM, Payne JM, Muhammad O, Ziady AG, Moen RC, Cooper MJ. Compacted DNA nanoparticles administered to the nasal mucosa of cystic fibrosis subjects are safe and demonstrate partial to complete cystic fibrosis transmembrane regulator reconstitution. Hum Gene Ther 2004;15:1255–1269. [PubMed: 15684701]

Kowalczyk TH, Pasumarthy MK, Gedeon C, Hyatt S, Payne JM, Muhammad O, Moen RC, Cooper MJ. Type of polylysine counterion influences morphology and biological function of condensed DNA. Molecular Therapy 2001;3:S259.

Lee D, Zhang W, Shirley SA, Kong X, Hellermann GR, Lockey RF, Mohapatra SS. Thiolated chitosan/DNA nanocomplexes exhibit enhanced and sustained gene delivery. Pharm Res 2007;24:157–167. [PubMed: 17103334]

Lewin AS, Drenser KA, Hauswirth WW, Nishikawa S, Yasumura D, Flannery JG, LaVail MM. Ribozyme rescue of photoreceptor cells in a transgenic rat model of autosomal dominant retinitis pigmentosa. Nat Med 1998;4:967–971. [PubMed: 9701253]

Li D, Yu H, Huang H, Shen F, Wu X, Li J, Wang J, Cao X, Wang Q, Tang G. FGF Receptor-mediated Gene Delivery using Ligands Coupled to Polyethylenimine. J Biomater Appl. 2007

Li SD, Huang L. Surface-modified LPD nanoparticles for tumor targeting. Ann N Y Acad Sci 2006;1082:1–8. [PubMed: 17145918]

Liu G, Li D, Pasumarthy MK, Kowalczyk TH, Gedeon CR, Hyatt SL, Payne JM, Miller TJ, Brunovskis P, Fink TL, Muhammad O, Moen RC, Hanson RW, Cooper MJ. Nanoparticles of compacted DNA transfect postmitotic cells. J Biol Chem 2003;278:32578–32586. [PubMed: 12807905]

Sarra GM, Stephens C, de Alwis M, Bainbridge JW, Smith AJ, Thrasher AJ, Ali RR. Gene replacement therapy in the retinal degeneration slow (rds) mouse: the effect on retinal degeneration following partial transduction of the retina. Hum Mol Genet 2001;10:2353–2361. [PubMed: 11689482]

Sesenoglu-Laird O, Svenson AG, Tyr O, Kowalczyk TH, Moen RC, Cooper MJ. Nuclease stability of siRNA and DNA condensed with PEGylated polylysine. Molecular Therapy 2007;15:S200.

Shirakawa T, Gotoh A, Terao S, Hinata N, Goda K, Tanaka K, Takenaka A, Hara I, Kao C, Gardner T, Chung L, Kamidono S, Fujisawa M. Long-term results of phaseI/II study of Ad-OC-TK/VAL gene therapy for the patients with metastatic or local recurrent prostate cancer. Molecular Therapy 2007;15:S279.

Sun W, Rosenblum R, Lee Z, Pagel M, Davis PB, Zaidy AG. Design and small animal imaging of PEG-stabilized DNA nanoparticles retargeted to the liver. Molecular Therapy 2007;15:S407.

Takahashi M, Miyoshi H, Verma IM, Gage FH. Rescue from photoreceptor degeneration in the rd mouse by human immunodeficiency virus vector-mediated gene transfer. J Virol 1999;73:781–816.

Thomas CE, Ehrhardt A, Kay MA. Progress and problems with the use of viral vectors for gene therapy. Nat Rev Genet 2003;4:34–58.

Tschernutter M, Schlichtenbrede FC, Howe S, Balaggan KS, Munro PM, Bainbridge JW, Thrasher AJ, Smith AJ, Ali RR. Long-term preservation of retinal function in the RCS rat model of retinitis pigmentosa following lentivirus-mediated gene therapy. Gene Ther 2005;12:694–701. [PubMed: 15660111]

Walsh M, Tangney M, O'Neill MJ, Larkin JO, Soden DM, McKenna SL, Darcy R, O'Sullivan GC, O'Driscoll CM. Evaluation of cellular uptake and gene transfer efficiency of pegylated poly-L-lysine compacted DNA: implications for cancer gene therapy. Mol Pharm 2006;3:644–653. [PubMed: 17140252]

Yurek DM, Fletcher-Turner A, Cooper MJ. Comepacted DNA nanoparticles effectively transfect brain cells *in vitro* and *in vivio*. Mol Ther 2005;11:253.

Zhang Y, Chen J, Zhang Y, Pan Y, Zhao J, Ren L, Liao M, Hu Z, Kong L, Wang J. A novel PEGylation of chitosan nanoparticles for gene delivery. Biotechnol Appl Biochem 2007a;46:197–204. [PubMed: 17147512]

Zhang Y, Zhang Y, Chen J, Zhang B, Pan Y, Ren L, Zhao J, Luo Y, Zhai D, Wang S, Wang J. Polybutylcyanoacrylate nanoparticles as novel vectors in cancer gene therapy. Nanomedicine. 2007b

Ziady AG, Gedeon CR, Miller T, Quan W, Payne JM, Hyatt SL, Fink TL, Muhammad O, Oette S, Kowalczyk T, Pasumarthy MK, Moen RC, Cooper MJ, Davis PB. Transfection of airway epithelium by stable PEGylated poly-L-lysine DNA nanoparticles in vivo. Mol Ther 2003;8:936–947. [PubMed: 14664796]