Medicon Pharmaceutical Sciences
Volume 2 Issue 3 April 2022
Review Article

Recent trends in Nanotech Delivery for Gene Therapy

Max Chase, Kevin Sneed and Yashwant Pathak*

Taneja College of Pharmacy, University of South Florida, Tampa Florida, USA

***Corresponding Author:** Yashwant Pathak, Taneja College of Pharmacy, University of South Florida, Tampa Florida, USA.

Received: February 23, 2022; **Published:** March 30, 2022

Abstract

Gene therapy is a fantastic new technology that has opened up a plethora of options to treat or prevent previously untreatable diseases. Gene therapy is the use of nucleic acids (either DNA or RNA) is used to manipulate the genetic information of a patient's cells. This is particularly helpful for diseases like Cystic Fibrosis where the ability to add a gene that makes the protein product the patient lacks or to just the gene itself is ideal. The addition of CRISPR-Cas for easier and more streamlined gene editing has also been a boon. However, the unfortunate truth is that despite decades of research, only a few gene therapies have made it to market. One of those reasons is the problems with carriers of gene therapy. For over three decades viral vectors were considered the go to for gene therapy carriers however they aggravate the immune system and can cause unwanted genotoxicity. This compromises their usefulness as a therapeutic treatment or preventative. So other methods of gene therapy carriers have been considered. Nanotech deliverers such as exosomes, gold nanoparticles, and others have shown great promise of being able to transport gene therapy with mitigated or no toxicity to the patient. This review will briefly go over both viral vector and nanotech non-viral vector alternatives to delivery of gene therapy.

Keywords: Gene therapy; nanotechnology; gene delivery; CRISPR; exosomes; viruses; magnetosomes

Introduction

Genetic mutations are one of the most common causes for disease and death worldwide. Through no fault other than birth, many people are predisposed towards cancer, CNS disorders, and autoimmune diseases. One of the best ways to prevent the onset of these diseases is to fix the root of the problem: the genes. Gene therapy in broad terms is the use modification of genes to either correct essential genes or to deactivate disease genes [1]. Through the introduction of exogenous nucleic acids. These exogenous nucleic acids can take the form of RNAs (such as siRNA, RNAi, miRNA), DNA (such as deoxy-oligonucleotides) or entire genes or gene segments [2]. Many diseases are based on a single gene mutation (monogenetic disorders). Toxic disorders such as Huntington's Disease, were misfolded proteins cause toxicity in the brain, can be solved with the use of gene therapy (such as small interfering RNA, or siRNA) to knockdown the over expression of the gene coding for said toxic protein. Gene therapy can also be used to add to something that the body does not make, for example calcium signaling proteins for someone with cognitive heart failure [3]. Ways to enact gene therapy have been varied such as the use of small interfering RNA (siRNA) to silence gene expression, insertion of plasmid DNA into the cell to act as a functioning gene, or editing the gene itself like with the CRISPR-Cas system [4].

However, the delivery of gene therapy is the one of the main issues that hold it back. Until we have the futuristic technology to just teleport the therapeutic DNA or RNA into the target cells, there has to be some way to bring the nucleic acids from the lab through the body and into the target cells. Nanotechnology has become a great help in that regard. Materials, both synthetic and organic, of between 1 and 100 nm in length, nanotechnology has shown in recent years how invaluable their unique properties are to developing new technology. The last thirty years have seen research into using viruses as vectors but they have their own issues to be discussed

in the viral vector section. To get around those other solutions have been researched, each with their own pros and cons. A successful carrier of gene therapy must be able to travel to its target cell/tissue, be specific enough to not offload its package onto unwanted cells, not cause an immune response, and finally not cause any unwanted mutagenesis [5]. Here in this review, I go over viral vectors and several alternatives that have shown up in recent years: exosomes, bacterial magnetosomes, and gold nanoparticles.

Naked DNA-The simplest solution

The first and simplest possibility to investigate the delivery of gene therapy would be to just use the therapeutic R/DNA and nothing else. No needing to deal with the hassle of modifying viral vectors, harvesting exosomes, or creating gold nanoparticles. One of the issues with naked nucleic acids are how they interact with the innate immune system. The immune system needs to detect self from non-self and then act accordingly, and one of the ways it uses to detect that is by the innate immunity's use of Pattern Recognition Receptors (PRRs) which recognize specific pathogen-associated molecular patterns (PAMPs). These PAMPs can include glycans, specific proteins, and nucleic acids. This activates the innate immune response, most likely causing inflammation and diminishing the potential benefits of the gene therapy [6]. The body also has a plethora of endonucleases in its physiological fluids and extracellular space, that would break down the R/DNA. After injection into mice, the half-life of plasmid DNA is around 10 minutes [7]. However, despite the setbacks there have been some successful attempts at using naked DNA, especially in a minicircle form. For example, in testing against Cystathionine β-synthase (CBS) deficiency, the naked minicircle DNA gene therapy it employed showed significant increases in gene expression at the targeted liver [8]. However, despite some success, finding a carrier for gene therapy that can successfully target and unload its package at the correct cells without causing toxicity is ideal.

CRISPR- a basic introduction

Within the last decade a great new innovation in gene therapy and gene editing has become widely used and has grabbed attention and should be given a mention here. This new technology is the CRISPR-Cas system standing for Clustered, Regularly, Interspaced, Short, Palindromic Repeats-CRISPER associated. CRISPER-Cas was first discovered in 1987, where it was found to give bacteria a sort of adaptive immunity to bacteriophages. Using this, if the bacteria survived a first bacteriophage infection could resist subsequent infections by that same bacteriophage. The system is comprised of 3 main components: the Cas protein (usually Cas9) which has endonuclease activity, single guided RNA (sgRNA), and a trans-activating crRNA (tracrRNA) which attracts Cas9. The sgRNA's first 10-12 nucleotides next to the proto-spacer adjacent unit (PAM) at the 3' end, called the seed sequence, directs the Cas9 protein in gene editing by binding to target site [9]. The Cas9 cleaves the DNA it is directed against by creating double strand breaks, neutralizing the threat and storing a portion of the insurgent DNA in the spacer sequence as a sort of memory so that the bacteria can protect itself from the phage in the future. In the 2010s, it was discovered that this anti-phage protection system bacteria had could be refitted to work on humans for gene editing. The process was streamlined compared to the natural version, using sgRNA to program the Cas9 protein [10]. This modified version of CRISPR uses 2 main components: a guide RNA (gRNA) and the Cas endonuclease (Usually Cas9). The former induces a conformational change in the latter, activating it and making it search out DAN that matches its PAM sequence. Upon finding its target, Cas will melt the bases upstream of PAM and try to pair it with the gRNA. If successful the Cas will induce double strand breaks 3-4 nucleotides upstream of PAM that will have to be repaired either by the error prone non-homologous end joining (NHEJ) DNA repair pathway or the less error prone Homology directed repair (HDR) pathway. Despite all the good press [11], CRISPR-Cas is not the be all end all and at the moment hasn't replaced other methods for gene therapy. The most pressing issue is that it literally was not made for mammalian cells, and has been shown to induce off target mutagenesis more often than it would in bacteria [12]. There have been fixes and modification but the technology is still not perfect.

Viral Vectors-The Classic

The classical way gene therapy components such as RNAi and CRSPR-Cas9 has been the use of modified viruses. Ove the course of billions of years, viruses have been specially designed by nature to target cells and unload a genetic package. In viral mediated gene therapy, viruses carry the nucleic acids and are modified to not replicate when after unloading their package. From recent clinical

trials, the most efficient viral vector from the adenovirus vectors, adeno-associated virus (AAV) vectors, and retroviral vectors (including lentiviral vectors). However, other viruses have been used as vectors as well such as the alphaviruses, flaviviruses, herpes simplex viruses and more [13]. Several viral vectors have gotten approval for clinical use. In 2012, Glybera (AAV) was approved for use in lipoprotein lipase deficiency [14]. In 2016 Strimvelis (retrovirus) was approved in Europe for treatment of severe combined immunodeficiency syndrome [15]. Kymriah (lentivirus based) and Yescarta (retrovirus) in 2017 were approved by the FDA to treat acute lymphoblastic leukemia and large B cell lymphoma, respectively [3, 16]. And in 2019 Zolgensma (AAV) was approved by the FDA to treat spinal muscular atrophy [17]. There are much more approved clinical viral based gene therapy vectors than the ones shown here, so suffice it to say that unlike the other vectors discussed here, viral vectors gene therapyhave already started to gain a strong foothold in clinical usage. Here is a basic overview of two of the more popular viral based gene therapies.

Adenovirus/AAVs

The most commonly used virus vector type is the adenovirus. They can infect a broad range of host cells, providing a short-term expression of gene of interest, and they have a packaging size of 7.5 kbp [18]. Unfortunately, the original version generated too high an immune response to be useful. Later variants have been modified to reduce that. However, most interestingly, as part of the research into using adenoviruses, AAV vectors were discovered. The AAV style of vectors themselves have some unique properties worth discussing. Its single strand DNA genome is comprised of the *rep* gene, the *cap* gene, and 2 inverted terminal repeats (ITR) that flank the other two [19]. *Rep* encodes proteins that aid in replication and assembly, while *cap* encodes capsid proteins (viral protein (VP) 1, 2 and 3) that later assemble into the viral capsid. The virion is composed of 60 VP capsids, each of which determine the general tropism and intercellular trafficking for the virus. These regions are also generally recognized by neutralizing antibodies. Change of the regions changes the transduction efficiency and ability to be recognized by neutralizing antibodies [20]. To infect cells, AAV virions bind to primary and secondary receptors to trigger endocytosis. The N terminuses' of VP1 and 2 are structurally shifted and exposed, allowing the virions entrance into the cell proper and to accumulate in the perinuclear region. The single stranded genome is release, which then becomes double stranded DNA template so the transgene can be transcribed and translated. The whole genome is approximately 4.7 kilobases (kb) in size [21], however the only necessary part for recombinant AAV (rAAV) propagation is the 145-base pair (bp) AAT IVRs which handle the induction of transgene expression and play an essential role in vector production and transduction [22]. This allows 96% of the genome can be replaced with a therapeutic string of nucleic acids. Infact, replacing the *rep* and *cap* genes with an expression cassette containing apromoter, therapeutic transgene, and a poly(A) tail is the basis of AAV vectors [23]. This allows a lot more flexibility when designing the attached gene therapy in the AAV vector. AAV vectors also have some other unique properties, such as the ability to be produced in excess of 10,000 particles per cell (making it easier to produce multitudes of stock), broad tropism, and low immunogenicity with no pathogenicity (mild innate and adaptive immune response at most) [24]; as well as long term transgene expression and rarely integrates into the host chromosome [19]. AAVs still have some limitations though: firstly, by the body having an immune response to multiple does of any specific serotype; the second being its limited storage capacity. Both these issues have been addressed over time: the first by using different serotypes each time, and the second by developing dual AV vectors. There is also the problem of random host genome integration, which can activate or inhibit endogenous gene expression. AAVs can still be improved more so, to be both more efficient and less immunologically activating, as discussed by Li and Samulski (2020) in their paper [25].

Retroviruses

The other major type of viral vector is retrovirus based viral vectors. Retroviruses don't just quickly use a cells machinery to replicate and then move one via lysis or budding, no instead they insert their own genome into the hosts DNA and lay dormant for a time. They do this by a process call reverse transcription, where the virus takes its ssRNA, reverse transcribes it into dsDNA, and uses the enzyme integrase to integrate it into the host DNA where it waits until it is activated by some stimulus. When activated it uses the host cell's machinery to build more virus particles. Simple retroviruses have only 4 fundamental genes: gag (Precursor proteins that become the matrix capsid and nucleocapsid proteins), *pro* (protease that processes the gagprecursors), pol (IN and reverse transcriptase (RT) enzymes) and *env* (surface (SU) and transmembrane (TM) domains glycoprotein of the viral envelope) [26]. Lentiviruses, a type

of retroviruses, have more complex genetic structure, with accessory genes allowing it have a more sophisticated method of regulation of viral functions. In any case, for gene therapy most of the viral genes are replaced with the therapeutic gene expression cassette and retaining only what is necessary for viral packaging, reverse transcriptase, and integration [27]. Retroviruses induce their package in a non-random process and prefer dividing cells for easier integration, though lentiviruses can access both dividing and non-dividing cells [28]. Retroviruses have a long history in gene therapy dating back to the 1980s, and more recently it has been used for Ex Vivo gene therapy of hematopoietic cells to permanently change those cells to combat diseases like severe combined immune deficiencies (SCIDs). These treatments can also be used to attend to other disorders like supplying a biotherapeutic protein or regenerate tissues [29].

Viral Issues

"These viruses seem great, what is the point of the rest of this paper?" One might ask. Well viruses are not perfect. Though viruses are a natural in targeting cells and delivering genetic packages, they are not designed to be safe to humans while doing so. The presence of a virus activates both the innate and adaptive immune response, as the components that make up a viral vector are still viruses and the body cannot make a distinction. The antiviral state the body becomes reduces the efficiency of these vectors and causing an inflammatory response [30]. Insertional mutagenesis is also possible which can have a number of effects, such as interference with transcription of neighboring genes as well as cellular transformation [31]. There is also the possibility of gain of function mutations, inadvertently causing the cell to become cancerous. In ex vivo style gene therapy that is not so much an issue since the tampered cells can be screened, but in vivo style gene therapy does not have this luxury. The opposite can happen, and the position of the gene insert can silence a gene possibly by interrupting its transcription in some way or epigenetically by the virus induced gene methylation disrupting the normal spread of methylation for protein recognition [32]. Either way, the virally inserted gene has created a new problem. For example, in animal testing mice injected with lentiviral vectors developed leukemia [33]. The transgene product may be a neo-antigen that is also the target of the immune system [30]. Several AAV/Ad issues were mentioned previously. Another factor to consider is that viral vectors, while still nano-sized, are still larger than many other nanotechnological methods for gene delivery, making it more difficult to move around in the body than say an exosome. A less toxic but still pressing issue is the difficulty of viral vector production [34]. With these and other issues, improving viral vectors while also finding alternatives seems to be the best option.

Exosomes-Small but Powerful

Exosomes are extracellular vehicles (EVs) excreted from cells that contain packages (proteins, DNA, RNA, lipids, amino acids, and metabolites) that are taken by other cells to effect cell function and behavior [35]. Between 30 and 100 nm in diameter, these EVs can escape rapid phagocytosis by phagocytes, pass through vascular endothelium, pass the blood brain barrier, and deliver drugs to target cells [36] The surface molecules on the exosome allow for its specific targeting, which scientists can engineer to increase this specificity [37]. This small size is a boon as well as a bane, as the small size prevents the usage of larger strands of nucleic acids like whole genes, but does easily allow for a smaller variety like small interfering RNA (siRNA) to be transported this way. One such example of this is by the team of Alvarez-Erviti et al., who used murine cells to create exosomes to deliver siRNA. The specific murine cells were immature murine dendritic cells, altered to express Lamp2- an exosomal membrane protein- which in turn was fused to a neuron specific RVG peptide. The resultant exosomes were purified and loaded with exogenous siRNA via electroporation. Using their miniscule bodies and lipid make-up these exosomes were able to go into neurons, microglia, and oligodendrocytes in the brain, without showing nonspecific uptake in other tissues. The content inside the exosomes were able to effectively bring in Cy5 labeled GAPDH siRNA in without issue, knocking out the specified gene. They later tested it with *BACE1*, a possible component of Alzheimer's disease, and showed significant knockdown in mice [37] showing *in vivo* capability.

Knocking down a gene or gene product is good, but how about the rewriting DNA that causes the disease? Normally gene editing tools like CRISPR-Cas9 are much too large for exosomes, however the team of Lin et al. have found a way through a hybrid liposome and exosome method. The team derived exosomes from the HEK293FT cell lines, which were then incubated with a mixture of liposomes

and pEGFP-C1 plasmids, allowing the encasement of the plasmid in the exosome-liposome fusion. Within this encapsulation, the plasmid was able to be successfully inserted into mesenchymal stem cells (MSCs). They then created a CRISPR interference and a cleavage system. The interference system targeted the mRunx2 gene, and used a sgRNA–dCas9 complex to repress the gene expression. The dCas9 physically blocked the RNA polymerase from binding during transcription elongation, while the the Runx2 gene's complementary sequence is recognized by the sgRNA. In an invitro experiment, this sgRNA-dCas9 system was encapsulated into the hybrid exosome and put into a mixture with murine MSC cells. Compared to the control group, the hybrid exosome was able to substantially increase expression of sgRNA and dCas9 while also significantly decreasing expression of the Runx2 gene. While impressive in being able to find a way to cram the needed components for CRISPR gene editing into an exosome hybrid, it is not without its faults. Mainly it is the liposome part of the exosome-liposome hybrid, as they can be toxic to the body. Should this fault be ironed out, then this hybrid exosome could be incredibly useful for clinical gene therapy and editing without the immune response that viral vectors can create [38].

Bacterial Magnetosomes- Farming Crystals

Bacteria have been around for billions of years and have become extremely diverse. This has let many species of bacteria develop unique pseudo-organelles, such as the magnetosome. *Magnetotacticbacteria* (or MTB), such as *Magnetospirillum gryphiswaldense*, have caught the eyes of researchers in recent years due to their unique pseudo-organelle, the magnetosome. An in-between the more organic side of nanotechnology and the more material, bacterial magnetosomes are iron oxide crystals wrapped inside a lipid bilayer. For bacteria, these crystals are used to orient themselves using the earth's magnetic field [39], but harvested these magnetosomes can be useful on their own. Unlike most magnets, magnetosomes are biocompatible, they're nanoscale small (40-120 nm) and their organic bilayer membranes can be modified to hold a variety of attached molecules including antibodies, nucleic acids, and small molecule drugs. Magnetosomes are easy to direct, it just takes the use of an external magnetic field to direct the crystal while it is in a body. That combined with their variability and ease of attachments on top of the lipid bilayer makes them very enticing as drug carriers, or as vehicles for therapeutic genetic materials [40]. For anticancer treatments, they can be used for tumor hyperthermia treatments using an altering magnetic field or laser [41]. However, as shown in the next paragraph, bacterial magnetosomes have potential as gene therapy delivery vehicles as well. Harvesting magnetosomes from bacteria has its own issues, as it requires growing the bacteria in a culture and environmentwhere they can be healthy and under the conditions to make the magnetosome, most likely using a batch fed method [42]. In addition, growth and production are also hampered by the presence of toxins needed to grow these bacteria, including toxic products as chelating agents and heavy metals. Fortunately, there have been some methods proposed to cut down on cost, complexity, and toxicity of growth while still keeping the similar production quality and quantity [43]. Should this keep improving, bacterial magnetosomes have a higher potential to be used more clinical settings.

A showing of the penitential of bacterial magnetosomes for gene therapy delivery and cancer treatment comes from by Wang et al, published in 2018. Beforehand the authors found that the antibacterial peptide, Cecropin B had potential as a tumor suppressant. Cecropin B causses defects in the membrane leading to the production of "pores". Chicken anemia virus VP3, named apoptin, had the potential to induce apoptosis by inducing G2/M arrest [44]. They found that a combination of these two genes could serve as an effective anti-tumor gene therapy [45]. They made a combination plasmid of the two genes, pVAX1-VA, with the bacterial magnetosome. This, along with, Lipofectamine 2000 as a comparison, showed successful transfection into HepG2 cells. After transfection, the cells showed the expression of cecropin B and apoptin, showing that the bacterial magnetosome is a suitable carrier for plasmid delivery. To determine transfection efficiency, the team used an already known plasmid, pIRES2-EGFP-VP3-ABPs, to directly visualize the transfection. It turned out the bacterial magnetosome-plasmid had a 3-fold higher transfection efficiency compared to Lipofectamine 2000. After ensuring transfection efficiency, the authors tested the ability of the bacterial magnetosome to increase pVAX1-VA induced apoptosis. Which the bacterial magnetosome-plasma showed to decrease HepG2 cell viability in a dose and time dependent manner, especially compared to the magnetosome on its own or by only using a vector. In comparison to a Lipofectamine 2000 carrier with the same plasma, the bacterial magnetosome-plasma had a 2-fold increase of early apoptotic cells. In checking the other section of the plasmid, the Cecropin B, the authors checked the mitochondrial caspases for cells transfected by the bacterial magnetosome-plasmid. The authors found an uptick in caspase 9 regulation, which is a key component of intrinsic apoptosis. For an in vivo experiment, the authors took

BALB/c Nude Mice with HepG2 induced tumors, and injected them with the bacterial magnetosome plasmid. The result was a striking amount of tumor inhibition, even compared to the control groups of PBS, Plasmid, or Vector control injected mice. The authors also reported no apparent toxicity [46]. This showed the potential of bacterial magnetosomes as an effective gene therapy option.

Lipofectamine- The Standard for everyone else

Lipofectamine deserves its own section. For lipid-based transfection reagents (TRs), the final transfection efficiency (TE) is rate limited by several biological barriers, including intracellular trafficking, cellular uptake, endosomal escape, and nuclear entry [47]. In the domain of transfection efficiency, Lipofectamine remains the "gold standard" for their high TE across a myriad of cell lines including the usually difficult to transfect ones. Lipofectamine follow lipoplex behavior when interacting with a cell, which follows three main phases. Phase 1 involves being bond to the cell and showing slow transport behavior. Phase 2 is that once within the cytosol, the lipoplex has an association with actin that results in anomalous and defined diffusion. In phase 3, active transport across along microtubules leads to accumulation around the nucleus. Lipofectamine avoids active transport by actin cytoskeleton as its only method of travel, instead also using Brownian motion, and thus avoiding lysosome degradation and increasing the possibility of releasing DNA into the cytosol [48]. This in turn leads to it's more optimal and efficient delivery. As can be seen in some of the following section, the main point of comparison to determine, an exosome's for example, efficiency will be against Lipofectamine. It also helps for lipofectamine that it has become a commercial product. Thermo Fisher Scientific provides several versions of lipofectamine, including Lipofectamine 2000 which is specifically mentioned in an "overwhelming amount" of publications according to the thermo fisher website, including some of the articles discussed in this paper [49].

Gold-Shiny and Effective

God nanoparticles (AuNPs) are 1-100 nm sized gold particles that have caught the attention of several industries, from medical to cosmetic. They have several unique properties that make them valuable: high biocompatibility, excellent catalytic activity, and optical characteristics [50]. For gene therapy vector transmission, gold nanoparticles can easily cross link with sulfhydryl (-SH) substances through Au-S bonds that allow them to manipulate their hydrophobicity and surface charge [50]. This allows gold nanoparticles to connect with CRISPR/Cas RNP. After administration, the payload can be released by thermal effect via a laser striking the AuNPs. In testing this against tumors, Wang et al (2018) also attached a cationic TAT peptide that guided the CRISP/Cas RNP to the nuclease of the cell, inhibiting the PLK-1 gene and thus inhibiting tumor growth. Another researcher Mount et al. (2017) modified Cas9 protein to have Glutamine at its N-terminus to balance out the protein normally positive charge. This let it link better cationic arginine-functionalized gold nanoparticles (ArgNPs) that were able to successfully deliver the Cas9 protein and sgRNA to the targeted nucleus and had a gene editing efficiency of 25-30% [52]. Another interesting factor of CRISPR-Gold Nanoparticles is that it can regulate the amount necessary per injection and reduce off target effects. Lee et al (2017) coated AuNPs with thiol modified DNA (thiol-DNA), that can hybridize with donor DNA and has loading affinity with Cas9, and encapsulating all that with PAsp (DET) creates CRISPR-Gold. This was used by Lee et al to repair a mutant dystrophin gene and reduce muscle fibrosis in X-linked muscular dystrophy (MDX) mice with a correction rate of 5.4% [53]. The authors of Lee et al. (2018) were able to use AuNP carriers to deliver gene therapy to mice, bypassing the blood brain barrier, and reduce the effects of fragile X syndrome in it [54]. Gold Nanoparticle have been seeing wide clinical use, so it will be interesting to see continued research in its capabilities in gene therapy in the future.

Conclusions

Unfortunately, this article is by no means comprehensive. Outside of the broad categories I have presented, there are many more tools for use in gene therapy. Even within those categories there is a deluge or example of researchers finding new ways to bring in a particular gene therapy to its target in a safer and more efficient manner. Gene therapy is incredibly complex, and after decades or proof of concepts of preclinical trials, there has been actual clinical use and commercialization. Now that gene therapy looks to becoming more mainstream in its use, it is more important than ever to make it as safe and efficient as possible. Naked DNA has been used with limited success, but it cannot survive on its own in the human body for long. For decades modified viruses were the target

of research, as they were already naturally gifted with travel, targeting, and payload dropping of their own or other genetic information. The main issues with these kinds of vectors are that the human body is evolved to recognize and attack viruses, and that viruses are not the most reliable in being able to hit only the specialized target with the gene therapy payload while also not causing some sort of Genotoxicity. Viral vectors are still being improved after decades and several have been approved for market. One other viable option is the use of exosomes. They are grown from the patient's cells making them biocompatible, and they are incredibly small even by the standards of the other nanocarriers allowing it to bypass the blood brain barrier. However, its incredible smallness prevents it from holding more than some RNAi like siRNA without modifications. Bacterial magnetosomes are an interesting choice as they have been shown to be biocompatible and able to attach gene therapy molecules on, with the added benefit of being able to better control its movement with a magnet. However, even with improvements on methods, harvesting BMs can be costly and possibly toxic. Lipofectamine is an interesting choice as it is not fully for clinical use but it is still a commercial product. Finally, gold nanoparticles are a synthetic solution that has seen some recent promise. Despite the drawbacks, the future for gene therapy has never been brighter and I for one cannot wait to see where it goes.

References

1. Humbert O, Davis L and Maizels N. "Targeted gene therapies: tools, applications, optimization". Critical reviews in biochemistry and molecular biology 47.3 (2012): 264-281.
2. Roma-Rodrigues C., et al. "Gene Therapy in Cancer Treatment: Why Go Nano?". Pharmaceutics 12.3 (2020): 233.
3. Dunbar CE., et al. "Gene therapy comes of age". Science 359.6372 (2018): eaan4672.
4. Hu B., et al. "Therapeutic siRNA: state of the art". Signal Transduction and Targeted Therapy 5.1 (2020): 101.
5. Papanikolaou E and Bosio A. "The Promise and the Hope of Gene Therapy". Frontiers in genome editing 3 (2021): 618346-618346.
6. Hull CM and Bevilacqua PC. "Discriminating Self and Non-Self by RNA: Roles for RNA Structure, Misfolding, and Modification in Regulating the Innate Immune Sensor PKR". Accounts of chemical research 49.6 (2016): 1242-1249.
7. Kawabata K., et al. "The fate of plasmid DNA after intravenous injection in mice: involvement of scavenger receptors in its hepatic uptake". Pharm Res 12.6 (1995): 825-830.
8. Lee HO., et al. "Treatment of Cystathionine β-Synthase Deficiency in Mice Using a Minicircle-Based Naked DNA Vector". Hum Gene Ther 30.9 (2019): 1093-1100.
9. Gaj T., et al. "ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering". Trends Biotechnol 31.7 (2013): 397-405.
10. Cong L., et al. "Multiplex genome engineering using CRISPR/Cas systems". Science 339.6121 (2013): 819-823.
11. Gupta D., et al. "CRISPR-Cas9 system: A new-fangled dawn in gene editing". Life Sci 232 (2019): 116636.
12. Fu Y., et al. "High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells". Nat Biotechnol 31.9 (2013): 822-826.
13. Lundstrom K. "Viral Vectors in Gene Therapy". Diseases 6.2 (2018): 42.
14. Büning H. "Gene therapy enters the pharma market: the short story of a long journey". EMBO Mol Med 5.1 (2013): 1-3.
15. Aiuti A., et al. "Gene therapy for ADA-SCID, the first marketing approval of an ex vivo gene therapy in Europe: paving the road for the next generation of advanced therapy medicinal products". EMBO Mol Med 9.6 (2017): 737-740.
16. Maude SL., et al. "Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia". New England Journal of Medicine 378.5 (2018): 439-448.
17. Keeler AM and Flotte TR. "Recombinant Adeno-Associated Virus Gene Therapy in Light of Luxturna (and Zolgensma and Glybera): Where Are We, and How Did We Get Here?". Annu Rev Virol 6.1 (2019): 601-621.
18. Schiedner G., et al. "Genomic DNA transfer with a high-capacity adenovirus vector results in improved in vivo gene expression and decreased toxicity". Nat Genet 18.2 (1998): 180-183.
19. Hastie E and Samulski RJ. "Adeno-associated virus at 50: a golden anniversary of discovery, research, and gene therapy success--a personal perspective". Hum Gene Ther 26.5 (2015): 257-265.

20. Xie Q., et al. "The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy". Proc Natl Acad Sci USA 99.16 (2002): 10405-10410.
21. Wu Z, Yang H and Colosi P. "Effect of genome size on AAV vector packaging". Mol Ther 18.1 (2010): 80-86.
22. Haberman RP., et al. "Novel transcriptional regulatory signals in the adeno-associated virus terminal repeat A/D junction element". J Virol 74.18 (2000): 8732-8739.
23. Wu Z., et al. "Optimization of self-complementary AAV vectors for liver-directed expression results in sustained correction of hemophilia B at low vector dose". Mol Ther 16.2 (2008): 280-289.
24. Samulski RJ and Muzyczka N. "AAV-Mediated Gene Therapy for Research and Therapeutic Purposes". Annu Rev Virol 1.1 (2014): 427-451.
25. Li C and Samulski RJ. "Engineering adeno-associated virus vectors for gene therapy". Nature Reviews Genetics 21.4 (2020): 255-272.
26. Vogt VM. "Retroviral Virions and Genomes". In J. M. Coffin, S. H. Hughes, & H. E. Varmus (Eds.), Retroviruses. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press (1997).
27. Schott JW., et al. 'Viral and Synthetic RNA Vector Technologies and Applications". Mol Ther 24.9 (2016): 1513-1527.
28. Buffone C., et al. "Nup153 Unlocks the Nuclear Pore Complex for HIV-1 Nuclear Translocation in Nondividing Cells". J Virol 92.19 (2018).
29. Naldini L. "Ex vivo gene transfer and correction for cell-based therapies". Nature Reviews Genetics 12.5 (2011): 301-315.
30. Shirley JL., et al. "Immune Responses to Viral Gene Therapy Vectors". Mol Ther 28.3 (2020): 709-722.
31. Aiuti A., et al. "The committee for advanced therapies' of the European Medicines Agency reflection paper on management of clinical risks deriving from insertional mutagenesis". Hum Gene Ther Clin Dev 24.2 (2013): 47-54.
32. Touw IP and Erkeland SJ. "Retroviral insertion mutagenesis in mice as a comparative oncogenomics tool to identify disease genes in human leukemia". Mol Ther 15.1 (2007): 13-19.
33. Cesana D., et al. "Uncovering and dissecting the genotoxicity of self-inactivating lentiviral vectors in vivo". Mol Ther 22.4 (2014): 774-785.
34. Bouard D., et al. "Viral vectors: from virology to transgene expression". Br J Pharmacol 157.2 (2009): 153-165.
35. Kalluri R and LeBleu VS. "The biology, function, and biomedical applications of exosomes". Science 367.6478 (2020).
36. Van den Boorn JG., et al. "SiRNA delivery with exosome nanoparticles". Nat Biotechnol 29.4 (2011): 325-326.
37. Alvarez-Erviti L., et al. "Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes". Nature Biotechnology 29.4 (2011): 341-345.
38. Lin Y., et al. "Exosome-Liposome Hybrid Nanoparticles Deliver CRISPR/Cas9 System in MSCs". Adv Sci (Weinh) 5.4 (2018): 1700611.
39. Simmons SL., et al. "South-seeking magnetotactic bacteria in the Northern Hemisphere". Science 311.5759 (2006): 371-374.
40. Kuzajewska D., et al. "Magnetotactic Bacteria and Magnetosomes as Smart Drug Delivery Systems: A New Weapon on the Battlefield with Cancer?". Biology 9.5 (2020): 102.
41. Beik J., et al. "Nanotechnology in hyperthermia cancer therapy: From fundamental principles to advanced applications". Journal of Controlled Release 235 (2016): 205-221.
42. Zhang Y., et al. "Semicontinuous culture of Magnetospirillum gryphiswaldense MSR-1 cells in an autofermentor by nutrient-balanced and isosmotic feeding strategies". Appl Environ Microbiol 77.17 (2011): 5851-5856.
43. Berny C., et al. "A Method for Producing Highly Pure Magnetosomes in Large Quantity for Medical Applications Using Magnetospirillum gryphiswaldense MSR-1 Magnetotactic Bacteria Amplified in Minimal Growth Media". Frontiers in bioengineering and biotechnology 8 (2020): 16-16.
44. Singh PK., et al. "Apoptin as a potential viral gene oncotherapeutic agent". Appl Biochem Biotechnol 176.1 (2015): 196-212.
45. Birame BM., et al. "Potentiation of Apoptin-induced apoptosis by Cecropin B-like antibacterial peptide ABPs1 in human HeLa cervical cancer cell lines is associated with membrane pore formation and caspase-3 activation". J Microbiol Biotechnol 24.6

(2014): 756-764.

46. Wang X., et al. "An enhanced anti-tumor effect of apoptin-cecropin B on human hepatoma cells by using bacterial magnetic particle gene delivery system". Biochem Biophys Res Commun 496.2 (2018): 719-725.
47. Suh J, Wirtz D and Hanes J. "Real-time intracellular transport of gene nanocarriers studied by multiple particle tracking". Biotechnol Prog 20.2 (2004): 598-602.
48. Cardarelli F., et al. "The intracellular trafficking mechanism of Lipofectamine-based transfection reagents and its implication for gene delivery". Scientific Reports 6.1 (2016): 25879.
49. Thermo Fisher Scientific, Lipofectamine 2000 Transfection Reagent.
50. Chen X, Li QW and Wang XM. "8 - Gold nanostructures for bioimaging, drug delivery and therapeutics". In N. Baltzer & T. Copponnex (Eds.), Precious Metals for Biomedical Applications (2014): 163-176.
51. Lévy R., et al. "Gold nanoparticles delivery in mammalian live cells: a critical review". Nano reviews 1 (2010).
52. Mout R and Rotello VM. "Cytosolic and Nuclear Delivery of CRISPR/Cas9-ribonucleoprotein for Gene Editing Using Arginine Functionalized Gold Nanoparticles". Bio Protoc 7.20 (2017).
53. Lee K., et al. "Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair". Nature Biomedical Engineering 1.11 (2017): 889-901.
54. Lee B., et al. "Nanoparticle delivery of CRISPR into the brain rescues a mouse model of fragile X syndrome from exaggerated repetitive behaviours". Nature Biomedical Engineering 2.7 (2018): 497-507.

Volume 2 Issue 3 April 2022