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Review Article

ACCREDITING GENE THERAPIES WITH NON-VIRAL LIPID NANOPARTICLES DELIVERY SYSTEM AND ITS RELATED PERTINENCE

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ABSTRACT

Gene therapy also called human gene transfer is defined as to cure a particular disease by relocate the genetic material. Lipid nanoparticles serve as the foremost advanced delivery method in consideration of genetic medication. Lipid nanoparticles are currently the advance and remarkable in non-viral delivery process for permissive the analytical potential of genetic medication. Genetic medication supported nucleic acids (RNA and DNA) because they contain uncommon remedial potential as just about any illness are often cured by silencing prophylactic cistron and damaged genes as well as convey a favourable protein. In this review, an attempt was made to clarify the progress in non-viral lipid nanoparticle delivery methods that have enticing properties. Therefore, the therapies supported nucleic acid polymers need refined delivery method to convey these macromolecules to the inside of target cells. Finally, we tend to address current application of lipid nanoparticles science as enforced to genetic medication.

Keywords: lipid nanoparticles, non-viral gene delivery, gene therapy, cationic liposomes

INTRODUCTION

The term gene expression is defined as the process by which information from a particular gene is used in the synthesis of a functional gene product. The principle behind lipid situated delivery method serve as a technology with an oversized knowledge domain and well- understood designing programme. Currently, ten lipid nanoparticles drugs that have obtained for restrictive approval for delivery of tiny molecule dope [1]. The key drawback is preventing the epidemic utilization of gene therapies supported nucleic acids (DNA or RNA) molecules are quickly degraded in biological fluids. Normally, specific organs like muscle or liver; naked DNA or RNA may be regionally injected and manufacturing high gene expression [2, 3]. Certainly, this type of strategies is proscribed to tissue that are simply attainable by direct injection like skin, where it is not applicable to integral gene delivery or is delusive for a commercial gene therapy. Here, we tend to specialize in non-viral delivery method that have benefits of simple producer, reduce immune responses, multi-dosing capabilities and adaptability of designs. The edges of non- viral delivery methods are lipid nanoparticles over 4 lipid nanoparticles small interfering RNA (siRNA) medication have entered the infirmary. Above all, gene conveyance vectors may be classified into viral and non-viral vectors [4, 5]. The viral vectors embrace the utilization of genetically built retrovirus, adenoviruses, adeno-associated and different viruses that are used for gene transfer procedures, the viruses having derived impeccable mechanisms through the series of evolution to deliver their genetic material into host cells. And another process of gene delivery is non-viral vectors that embrace the cationic liposomes complexes either victimization of cationic lipids like DOGS (octadecylamidoglycylspermine) or cationic polymer like PLL (poly (L-lysine) [4-8]. Non-viral vectors are more popular over viral vectors as a result of they are non-immunogenic in nature.

RNAi is a present method of gene expression and regulation present in plants and mammalian cells. From a curative purpose of view, RNAi offers the possibility to gene silencing in a very sequence – specific manner. This method is predicted on siRNA that are short dsRNAs of 21 nucleotides long and able to arbitrate site specific cleavage and degraded the targeted mRNA [9].

LIPID NANOPARTICLES

The foremost process in lipid nanoparticles are clinically advanced non-viral gene delivery methods. The improvement and benefit of genetic drugs is safely and adequately deliver nucleic acids (DNA and RNA) with the help of lipid nanoparticles. Genetic drugs has many alternative application like gene editing, expeditious vaccine progress and treatment of scarce hereditary diseases and all of that are typically inhibited by disorganization of nucleic acid delivery. Enhanced diffusion into tissue to delivery analysis, mediocre cytotoxicity and immunogenicity and immense nucleic acid insulation adaptability are main characteristics cause for lipid nanoparticles admirable bidder for nucleic acid delivery.

Lipid nanoparticles in gene therapy

Solid lipid nanoparticles were different from liposomes in debt to their large capability to defend the active additive from destruction and circumstances of inflect the discharged profile [10]. For gene transmission process, lipid based methods are broadly sanctioned, on account of well endured physiological lipids and also accepted in pharmaceutical for human beings [11]. Besides, for long term adherence, commercial sterilization and lyophilized procedures, solid lipid nanoparticles accomplished with separate technique auspiciously executed in pharmaceutical companies [12-14]. A sheet of surfactants covered the solid lipid core in solvent dispersion and solid lipid nanoparticles must be smaller than 500 nm in diameter and 120 nm or less is standard size

for in vivo condition. According to gene therapy application, the surfactants are normally positively charged to get the cationic solid lipid nanoparticles that are attached electrostatically to nucleic acids [15]. If the nucleic acid is formerly attached to a cationic ingredient, then anionic solid lipid nanoparticles are also able to induce transfection [16, 17]. In point of fact, a big concern for transfection in non-viral vectors is clemency of nucleic acid [18]. Nanostructured lipid carriers have been also examined as gene transmission methods [19] and also elaborated the contingency to affix electrostatically nucleic acids while encapsulating a lipophilic medication in the origin [20-22].

Various snags in gene therapy by lipid nanoparticles

In lipid nanoparticles, the probability of being operative in form to swamped all these barriers in the transfection process. The nucleic acid is attached to cationic solid lipid nanoparticles electrostatically and defends from environmental deterioration and this has been broadly manifested in vitro by electrophoresis in agarose gel [15, 23, and 24]. Solid lipid nanoparticles depend on both cationic lipid as well solid lipid cationic lipid combinations for the binding capacity. The cationic one balances the crystalline state that is induces the large stability than the use of cationic lipids that dissolve in the solid lipid. Therefore, the surfactants engaged for solid lipid nanoparticles production don't seems to be influence the nucleic acid binding capacity [25]. Moreover, the enormous contraction of nucleic acids is abominable because the acquittal of active molecule would be defined resulting in poor transfection adequacy [15]. Another method to protect nucleic acids is the encapsulation into the nanoparticles depth and targeted on siRNA insulation into NLCs [26, 27]. After all, nucleic acids are hydrophilic in nature; siRNA needs to associate with hydrophobic molecules that can be indulged with lipid core components. Xue et al. [27] attached the siRNA to a commixture of oleic acid with poly-[arginine, tryptophan] and Lobovkina et al. [26] applied the hydrophobic molecules pairing approach, that consist of a tight complex of siRNA and the cationic lipid (1,2- dioleoyl-3-trimethylammonium-propane (DOTAP). Like poly (ethylene glycol) (PEG) flexibility [15, 28] shielding of cationic charges with water-soluble, flexile polymer and this approach also beneficial for the transmission of nucleic acids by different executions.

Cell membrane and trafficking is another barrier in gene therapy transmission by lipid nanoparticles. If a particular target isn't bound on the cationic lipid nanoparticles, the attachment to the cell membrane occurs through electrostatic interaction between the positively and negatively charges of the cell membrane [29]. Endocytosis is main entry mechanism of solid lipid nanoparticles based non-viral vectors. Many more mechanism of endocytosis has been explained such as phagocytosis, clathrin dependent/independent endocytosis [30]. Recently, nanostructured lipid carriers have been enhancing with transferrin (TfNLCs) for gene therapy active targeting to lung cancer cells [19, 21]. After treatment with TfNLCs, results showed more gene expression in lung cancer cells in correlation to non-enhanced nanostructured lipid carriers. Other targeting approach reported for solid lipid nanoparticles based nucleic acid delivery is the polysaccharide hyaluronic acid, which gets attached to the CD44 receptors [31]. In intracellular trafficking, the desertion of nucleic acids from the endosomes before degeneration in lysosomal vesicles is compelling for efficient transfection. The agility of transmission to the endolysosomal compartments depends on the entry mechanism [32-34] and it's important to decisive the specific endocytic pathway used in each particular target cell.

Lipid nanoparticles pattern for nucleic acids conveyance

For lipid-based convey system for nucleic acids are required some design features like standard particle size, [1, 35] almost 100% encapsulation abilities, fast manufacturing system and low surface charge to diminish synergy with serum proteins. Therefore, the deliver cargo to the cytoplasm of object cell, this design must implement active and scalable entrapment of nucleic acids as well as capability of the resulting lipid nanoparticles. To accomplish encapsulation of negatively charged polymers, cationic polymers are obligatory.

Cationic liposome used in non-viral gene therapy

Earlier, to produce lipid nanoparticle system confide on entrapment through static methods using zwitter ion lipids that used electrostatic equity to get entrapment. Such things showed destitute deception efficiencies [36] and limited transfection efficacy [37]. When DOTMA was mixed with DOPE (dioleoylphosphatidylethanolamine) then it's accredit more productive entrapment and transfection efficacy through formation of complexes by mixing with DNA in solutions known as lipoplexes. Lipoplexes are erratic and characterized by wide area distribution ranging from sub-micron scale to some micron. Lipofectamine reagent type of lipoplexes has found considerable utility for in vitro transfection [38, 39]. During cationic lipids and lipid nanoparticles, genetic drug containing a cationic lipid used a detergent dialysis technique for lipid nanoparticles formulation. To associate with negatively charged polymers, high levels of nucleic acid polymers in lipid nanoparticles systems intuitively need high scales of cationic lipids. However, in vivo use of cationic lipids has inherent limitations. Lipid nanoparticles with a momentous surface charge consume serum proteins and are readily cleared from the circulation [1]. Moreover, cationic lipids conjointly with endogenous anionic lipids to form non-lamellar phase structures [40]. Cationic lipids also needed to get efficient loading of nucleic acid polymers into lipid nanoparticles.

Cationic lipids and Ethanol loading process

The dialysis loading process is very prominent for developing homogenous lipid nanoparticles and the ethanol loading process were concoct for use in consolidation with ionisable cationic lipids. Initially, lipids dissolved in ethanol and adding this solution to antisense oligonucleotides (ASO) in pH 4 buffer [41]. The resulting fragments were ejected to achieve a homogenous population of 100 nm lipid nanoparticles followed by dialysis to abolish solvent and optimize the pH. This method emanate in entrapment efficiencies of 80% for a lipid concentration. And another emphasis of the ethanol loading process indulged the avail of an instinctive vesicle formation process used the Balzri and Korn ethanol injection mechanism [42]. In this mechanism, the desired lipids are mixed in ethanol at exact ratios while pDNA is mixed in an acidic medium buffer. The whole solutions are then swiftly mixed by a T-junction [43]. And then emanate nanoparticles were put through another T-junction to dilute the ethanol concentration. An improvement to this loading protocol, the entrapment was determined to be increasing around 80% after second dilution. And to achieve a final concentration, it relied on the combination of two mixing steps into a single step [44]. These processes achieve adequately fully encapsulation and some advantage of being gently as needed by nucleic acid polymers.

Pertinences of gene therapy

According to clinical application, it is based on utilization of viral vectors and is presently restricted to serious diseases that don't have any cure. Even the auspicious strategy that gene therapy presumes for many diseases, its inherent risk still makes decisive studies to increase characteristics and effectiveness

concerns. All the while, vital progresses are continuing with non-viral vectors systems [45], culminating in additional efficient and biocompatible formulations that achieves into in vivo studies and aegis future clinical trials. The foremost studied in gene medication to be cured with gene therapy and lipid nanoparticles embody ocular disease, infectious diseases and cancer. In this review, we present here some paradigm of the lipid nanoparticles for the treatment of a number of these diseases by gene therapy.

Transmissible or infectious disease

Nowadays gene therapy used in most of the infectious disease as well as bacterial, viral and parasite infection further. Infectious diseases are within top three ranking of clinical application in gene therapy [45]. The purpose of gene therapy is depiction as a treatment if any bug is established or any vaccine to stop the infection process. For instances, the utilization of genes to stop the replication of a virus or any gene that arouse the immune system like gene encoding foreign particles or any non-functional protein part of a virus. iRNA to knockdown genes of the infectious agent is additionally doable. siRNA and shRNA has been advised to probably cure infections.

Another auspicious method is that the use of RNAi as prevention of infections [46]. Last few years, the pertinence of lipid nanoparticles for the cure of infectious diseases by gene therapy is rising. Hepatitis C virus infection may be a big health threat across the world [47]. Torrecilla et al established solid lipid nanoparticles based vectors for the cure of Hepatitis C viral infections. The vectors enclosed with an expression plasmid [shRNA74] which is stop the interior ribosome entry site (IRES) of Hepatitis C viral infection that is obligatory for the replication process.

Currently, Human immunodeficiency virus (HIV) is a major public health problem, though effective cure with anti-retroviral drugs will control the virus replication but there is no treatment for HIV infection and more brilliant methods should be developed. In recent times, solid lipid nanoparticles are considered as a method for HIV DNA vaccine transmission [48]. Solid lipid nanoparticles showing multiple epitope gag protein (pHIS-HIV-hugag) were ready with high homogenization techniques and assess in vitro in hela cells. Nevertheless, the solid lipid nanoparticles had capability to transfect and ought to be reformulated to enhance its potential for transfection.

Ocular disease

Gene therapy has eventuated in a great pledge for the cure of ocular (sight) disease affecting the retina as well cornea [49]. Eye has a very good anatomy and it's very accessible and easily to examined. Earlier, a research group showed the expression of EGFP in sight related disease of rats examine with a solid lipid nanoparticles based non-viral vector addressing the reporter gene pCMS-EGFP [50]. After examination, they found the expression of GFP in different types of cells depending on that authority. It was a great response in retinal cells when intravitreal injection was engaged but in RPE cells, the protein expression was less. Later, the vectors were capable to transfect RPE cells as well as photo receptors after injecting in sub-retinal. In recent times, eleven evaluated non-viral vectors based on solid lipid nanoparticles, protamine and hyaluronic acid as carrier of RS1 gene that code for the protein retinoschisin. The most unfavourable result in sight activity is seen when extensive schisis that indulge the central retina [51].

At present for low vision aids, there is no treatment based on gene therapy application [52]. The vector used in RS1h-deficient mice, and 2 weeks later sub-retinal or intravitreal injection and retinoschisin expression was found with different retinal layers and it was kept for 2 months after sub-retinal handling. And the structural analogy of the cured Rs1h-deficient visuals showed incomplete recovery of the retina related to the elongation of retinoschisin. These examination serve as the most progressive work in which solid lipid nanoparticles consisting genetic material are able to recrudesce a schism and therefore to be an authentic approach for gene therapy.

Lysosomal degradation process or lysosomal storage disorders

Another group of harmful diseases due to collapse in lysosomal abasement process including around many different disorders, most of them uncommon, hereditary and autosomal recessive diseases. Normally, lysosomal disorders are incurable and progressive disorders that can be present with a wide spectrum of harshness and signs. They affect the multiple organs at same time including the skeletal system, liver, heat and lungs and mainly central nervous system which is mainly affected by this disorder. Lysosomal disorders are most common origin or cause of paediatric neurodegenerative disease [53]. Treatment of lysosomal disorders with gene therapy by using lipid nanoparticles is deficient. In recent times, vectors based on solid lipid nanoparticles to cure Fabry disease caused by a deficiency of lysosomal enzyme i.e. α-galactosidase A (α-Gal A). After that, the capability of vectors is increased the α-Gal activity was confirmed in IMFE1 cells [54].

Cancer therapy

In recent times, a major development has been contrived in evolving cancer gene therapy that might be capable to cure the cancer. Cancer application is the preeminent indication of gene transmission across the world [41]. Mostly to cure the cancer disease, viral vectors always first choice for treatment and for non-viral vectors mainly lipid nanoparticles is limited. But [55] cationic solid lipid nanoparticles were developed to encapsulate the signal transducer and act as an activator of transcription i.e. STAT3, a member of STAT family that recognized as cancer causing gene or oncogene. The barricade of the STAT3 pathway resulted in cell death. Recently [56] solid lipid nanoparticles consisting EGFP plasmid and doxorubicin were produced to make a multifunctional delivery system that specifically points to lung cancer cells, to improve the efficiency of cancer therapy. pEGFP and doxorubicin loaded solid lipid nanoparticles were prepared independently and then mixed and covered with transferrin-consisting receptors. The vectors in human alveolar adenocarcinoma cell line (in vitro transfection efficacy) A549 cells was accurately higher than obtained with new pEGFP and blank solid lipid nanoparticles. Human (non-small) lung cancer cells [57] (H1299 cells) evaluated transfection efficiency and p53 tumour suppressing gene has been found in solid lipid nanoparticles. Additionally, the efficacy re-establishment of wild type p53 gene in lung cancer cells restored by the apoptotic pathway. Later, the vector containing pp53-EGFP and mRNA expression of GFP was protracted in lung, liver and spleen cells.

CONCLUSION

Viral vectors are static efficient methods for gene transmission, but in terms of immunogenicity and expense, have encouraged scientists to focus on alternative methods for gene transfer such as lipoplexes or polyplexes. According to non-viral transfection used for in vitro applications, cationic solid lipid nanoparticles have been developed few years back as a substitute carrier for DNA delivery and other advantages over non-viral vectors. And although cationic lipid based solid lipid nanoparticles possess many benefits like nucleic acids transfer agents in cell culture. Additionally, cationic solid lipid nanoparticles are created by using well-tolerated ingredients already approved for pharmaceutical

application in humans. The cationic solid lipid nanoparticles or nucleic acids compounds can defend nucleic acids from enzymatic destruction and transfer the nucleic acids into cells by cooperating with negatively charged cell membrane. Lipid nanoparticles based vectors represent another system to viral vector system due to safety and improvement concerns. Although they can be simply developed at big scale, can be sterilized and also stable in biological fluids as well as in storage purposes.

Currently, nanotechnologies are focused on the efficacy transmission of a therapeutic to the specific target sites, a next aim might enhance the functionality by knowing gene combination and transfer them at a given time. The further success of cationic solid lipid nanoparticles will depend on their capability to cross the biological barricade and pointing a specific cell type in vivo. It states that the success of gene delivery system or gene therapy obligate to be in higher organism disease models before it will harmful to human beings. Even though many barriers, the gene therapy has wide clinical application and must ongoing to move ahead developments in transmission strategies.

REFERENCES

1. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. Adv Drug Delivery Rev 2013; 65:36–48
2. Walther W, Stein U, Voss C et al. Stability analysis for long-term storage of naked DNA: impact on nonviral in vivo gene transfer. Analytical Biochem 2003; 318:230-5
3. Shi F, Rakhmilevich AL, Heise CP et al. Intratumoral injection of interleukin-12 plasmid DNA, either naked or in complex with cationic lipid, results in similar tumour regression in a murine model. Mol Cancer Therapy 2002; 1:949-57
4. Morille M, Passirani C, Vonarbourg A et al. Progress in developing cationic vectors for non-viral systemic gene therapy against cancer. Biomaterials 2008; 29:3477-96.
5. EL-ANEED A. An overview of current delivery systems in cancer gene therapy. J Control Release 2004; 94:1-14
6. Bhavsar MD, Amiji MM. Polymeric nano and micro particle technologies for oral gene delivery. Expert Opin Drug Deliv 2007; 4:197-213.
7. Jin S, Ye K. Nanoparticle-mediated drug delivery and gene therapy. Biotechnology Prog 2007; 23:32-41
8. Kaneda Y, Tabata Y. Non-viral vectors for cancer therapy. Cancer Sci 2006; 97:348-54
9. Lares MR, Rossiand JJ, Ouellet DL. RNAi and small interfering RNAs in human disease therapeutic applications. Trends Biotechnology 2010; 28:570–79.
10. Swathi G, Prasanthi NL, Manikiran SS, Ramarao N. Solid Lipid Nanoparticles: Colloidal Carrier Systems for Drug Delivery. IJPSR 2010; 1:1-16.
11. Schwarz C, Mehnert W, Lucks JS, Muller RH. Solid lipid nanoparticles for controlled drug delivery. I. Production, characterization and sterilization. J Control Rel1994; 30:83–96.
12. Muller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev 2002; 54:131–55.
13. Del Pozo-Rodríguez A, Solinís A, Gascón AR, Pedraz JL. Short and long term stability study of lyophilized solid lipid nanoparticles for gene therapy. European J Pharm and Biopharm 2009; 71:181–89.
14. Del Pozo-Rodríguez A, Delgado D, Gascon AR Solinís MA. Lipid nanoparticles as drug/gene delivery systems to the retina. J Ocular Pharmacol Ther. 2013; 29:173–88.
15. Del Pozo Rodríguez A, Delgado D, Solinís MA, Gascon AR, Pedraz JL. Solid lipid nanoparticles: formulation factors affecting cell transfection capacity. Int J Pharma 2007; 339:261-68.
16. Ye J, Wang A, Liu C, Chen Z, Zhang N. Anionic solid lipid nanoparticles supported on protamine/DNA complexes. Nanotechnology 2008; 19:285-90.
17. Yuan H, Zhang W, Du YZ, FQ Hu. Ternary nanoparticles of anionic lipid nanoparticles/protamine/DNA for gene delivery. Int J Pharm 2010; 392:224-31.
18. Sakurai F, Inoue R, Nishino Y, Okuda A, Matsumoto O, Taga T. Effect of DNA/liposome mixing ratio on the physicochemical characteristics, cellular uptake and intracellular trafficking of plasmid DNA/cationic liposome complexes and subsequent gene expression. J Control Release 2000; 63:255–69.
19. Han Y, Li Y, Zhang P, Sun J, Li X, Sun X, Kong F. Nanostructured lipid carriers as novel drug delivery system for lung cancer gene therapy. Pharm Dev Technol 2016; 21:277-81.
20. Han Y, Zhang Y, Li D, Chen Y, Sun J, Kong F. Transferrin-modified nanostructured lipid carriers as multifunctional Nano medicine for co delivery of DNA and doxorubicin. Int J Nanomedicine 2014; 9:107-16.
21. Shao Z, Shao J, Tan B, Guan S, Liu Z, Zhao Z et al. Targeted lung cancer therapy: preparation and optimization of transferrin-decorated nanostructured lipid carriers as novel nanomedicine for co-delivery of anticancer drugs and DNA. Int J Nanomedicine 2015; 10:1223-33.
22. Chen Z, Lai X, Song S, Zhu X, Zhu J. Nanostructured lipid carriers based temozolomide and gene co-encapsulated nanomedicine for gliomatosis cerebri combination therapy. Drug Deliv 2015; 2:1-5.
23. Radaic A, De Paula E, De Jesus MB. Factorial Design and Development of Solid Lipid Nanoparticles (SLN) for Gene Delivery. J Nanosci Nanotechnology. 2015; 15:1793-1800
24. Montana G, Bondì ML, Carrotta R, Picone P, Craparo EF, San Biagio PL et al. Employment of cationic solid-lipid nanoparticles as RNA carriers. Bioconjug Chem. 2007; 18:302-8
25. Doktorovova S, Shegokar R, Rakovsky E, Gonzalez-Mira E, Lopes CM, Silva AM et al. Cationic solid lipid nanoparticles (cSLN): structure, stability and DNA binding capacity correlation studies. Int J Pharm 2011; 420:341-9.
26. Lobovkina T, Jacobson GB, Gonzalez-Gonzalez E, Hickerson RP, Leake D, Kaspar RL et al. In vivo sustained release of siRNA from solid lipid nanoparticles. ACS Nano. 2011; 5:9977-83.
27. Xue HY, Wong HL. Tailoring nanostructured solid-lipid carriers for time-controlled intracellular siRNA kinetics to sustain RNAi-mediated chemo sensitization. Biomaterials 2011; 32:2662-72.
28. Schiffelers RM, De Wolf HK, Van Rooy I, Storm G. Synthetic delivery systems for intravenous administration of nucleic acids. Nanomedicine-UK 2007; 2:169–81.
29. Del Pozo-Rodríguez A, Delgado D, Solinís MA, Gascón AR, Pedraz JL. Solid lipid nanoparticles for retinal gene therapy: transfection and intracellular trafficking in RPE cells. . Int J Pharm. 2008; 360:177-83.
30. Ruiz de Garibay AP, Solinís MA, Rodríguez-Gascon A, Ganjian H, Fuchs R. Role of endocytic uptake in transfection efficiency of solid lipid nanoparticles-based nonviral vectors. J Gene Med. 2013; 15:427-40.
31. Almalik A, Day PJ, Tirelli N. HA-coated chitosan nanoparticles for CD44-mediated nucleic acid delivery. Macromol Biosci. 2013; 13:1671–80.
32. Rejman J, Bragonzi A, Conese M. Role of clathrin- and caveolae-mediated endocytosis in gene transfer mediated by lipo- and polyplexes. Mol Ther. 2005; 12:468-74.
33. Van der Aa MA, Huth US, Häfele SY, Schubert R, Oosting RS, Mastrobattista E et al. Cellular uptake of cationic polymer-DNA complexes via caveolae plays a pivotal

role in gene transfection in COS-7 cells. Pharm Res. 2007; 24:1590-98.
34. Ur Rehman Z, Hoekstra D, Zuhorn IS. Protein kinase A inhibition modulates the intracellular routing of gene delivery vehicles in HeLa cells, leading to productive transfection. J Control Release. 2011; 156:76-84.
35. Ernsting MJ, Murakami M, Roy A, Li SD. Factors controlling the pharmacokinetics, bio distribution and intratumoral penetration of nanoparticles. J Control Release 2013; 172:782–94.
36. Fraley R, Subramani S, Berg P, Papahadjopoulos D. Introduction of liposome-encapsulated SV40 DNA into cells. J Biol Chem. 1980; 255:10431–35.
37. Fraley R, Straubinger RM, Rule G, Springer EL, Papahadjopoulos D. Liposome-mediated delivery of deoxyribonucleic acid to cells: enhanced efficiency of delivery related to lipid composition and incubation conditions. Biochemistry 1981: 20:6978–87.
38. Filion MC, Phillips NC. Toxicity and immunomodulatory activity of liposomal vectors formulated with cationic lipids toward immune effector cells. Biochim Biophys Acta 1997; 1329:345–56.
39. Lappalainen K, Jaaskelainen I, Syrjanen K, Urtti A, Syrjanen S. Comparison of cell proliferation and toxicity assays using two cationic liposomes. Pharm Res 1994; 11:1127–31.
40. Hafez IM, Maurer N, Cullis PR. On the mechanism whereby cationic lipids promote intracellular delivery of polynucleic acids. Gene Ther. 2001; 8:1188–96.
41. Semple SC, Klimuk SK, Harasym TO, Dos Santos N, Ansell SM, Wong KF et al. Efficient encapsulation of antisense oligonucleotides in lipid vesicles using ionisableamino lipids: formation of novel small multilamellar vesicle structures. Biochim Biophys Acta 2001; 1510:152–66.
42. Jeffs LB, Palmer LR, Ambegia EG, Giesbrecht C, Ewanick S, MacLachlan I. Extrusion free method for efficient liposomal encapsulation of plasmid DNA. Pharm Res 2005; 22:362–72.
43. Batzri S, Korn ED. Single bilayer liposomes prepared without sonication. Biochim Biophys Acta 1973; 298:1015–19.
44. Hirota S, De Ilarduya CT, Barron LG, Szoka FC. Simple mixing device to reproducibly prepare cationic lipid-DNA complexes (lipoplexea). Biotechniques 1999; 27:286–90.
45. Belliveau NM, Huft J, Lin PJ, Chen S, Leung AK, Leaver TJ et al. Microfluidic synthesis of highly potent limit-size lipid nanoparticles for in vivo delivery of siRNA. Mol Ther Nucleic Acids 2012; 1: 37
46. Jon Wiley, Sons. Gene Therapy Clinical Trials Worldwide. Journal of Gene Med. 2015; 3
47. Torrecilla J, Rodríguez Gascon A, Solinís MA, Del Pozo-Rodríguez A. Lipid nanoparticles as carriers for RNAi against viral infections: current status and future perspectives. Biomed Res Int. 2014; 11-4
48. Tai AW, Chung RT. Treatment failure in hepatitis C: mechanisms of nonresponse. J Hepatol. 2009; 50: 412-20.
49. Asasutjarit R, Lorenzen SI, Sirivichayakul S, Ruxrungtham K, Ruktanonchai U, Ritthidej GC. Effect of solid lipid nanoparticles formulation compositions on their size, zeta potential and potential for in vitro pHIS-HIV-hugag transfection. Pharm Res. 2007; 24:1098-1107.
50. Solinís MA, Del Pozo Rodríguez A, Apaolaza PS, Rodríguez Gascon A. Treatment of ocular disorders by gene therapy. Eur J Pharm Biopharm. 2015; 95:331-42.
51. Delgado D, Del Pozo-Rodríguez A, Solinís MA, Aviles-Triqueros M, Weber BH, Fernández E et al. Dextran and protamine-based solid lipid nanoparticles as potential vectors for the treatment of X-linked juvenile retinoschisis. Int J Pharm. 2012; 23:345-55.
52. Audo I, Mohand-Saïd S, Sahel JA, Holder JE, Moore AT. X-Linked Retinoschisis inherited Chorioretinal Dystrophies. Springer-Verlag, Berlin Heidelberg.2014; 22:383-90.
53. Molday RS, Kellner U, Weber BH. X-linked juvenile retinoschisis: clinical diagnosis, genetic analysis, and molecular mechanisms. Prog Retin Eye Res.2012; 31: 195–212.
54. Coutinho MF, Alves S. From rare to common and back again: 60years of lysosomal dysfunction. Mol Genet Metab.2016; 117:53-65.
55. Ruiz de Garibay AP, Solinís MA, Rodríguez Gascon A. Gene Therapy for Fabry Disease: A Review of the Literature. Bio Drugs 2013; 27:237–46.
56. Ma Y, Zhang X, Xu X, Shen L, Yao Y, Yang Z et al. STAT3 Decoy Oligodeoxynucleotides-Loaded Solid Lipid Nanoparticles Induce Cell Death and Inhibit Invasion in Ovarian Cancer Cells. PLoS One.2015; 20-31
57. Han Y, Zhang P, Chen Y, Sun J, Kong F. Co-delivery of plasmid DNA and doxorubicin by solid lipid nanoparticles for lung cancer therapy. Int J Mol Med.2014; 34:191-96.