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Nanoparticle as Delivery System: A New Genetic Engineering Approach Review

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Abstract: ***In the last only some years, lots of groups have reported the use of nanoparticles to complex and deliver viral vectors (e. g., adenoviruses, retroviruses) and nucleic acids, foremost to the appearance of new approaches known as magnetofection and the ranostics. Viral vectors are further competent than non - viral vectors for DNA delivery but may present a significant danger to patients, while non - viral carriers are naturally safer than viral carriers. Also, in contrast to the viral gene delivery systems, the non - viral carriers are usual to be less immunogenic, with simple preparation and a possible versatile outside alteration.***

Keywords: Nanoparticle; genetic; gene; virus; engineering

1. Introduction

These days, gene delivery for therapeutic objects is measured one of the mainly capable strategies to cure equally the genetic and acquired diseases of human. The design of resourceful gene release vectors possessing the high transfection efficiencies and low cytotoxicity is careful the major face for delivering a target gene to exact tissues or cells. By in view of the mentioned issues, the choice of a appropriate method for DNA delivery to the targeted cells beseems very important at the point of receipt suitable genes. Although gene therapy can be passed out using naked DNA into the target cells, having negative nature of cellular membrane and negative accuse of great DNA molecules, the nucleic acid - based therapeutics cannot cross cellular membranes by simple passive diffusion methods. Thus, to make possible the move of DNA molecules into a cell, the survival of a vector is necessary [Ponder et al.2002, Philippi et al.2010]. Viral and non - viral vectors, two mainly important types of vectors for gene delivery, are currently being utilized in clinical trials at related levels.

In gene delivery, it is moderately general to go after biomimetic approaches. Biological systems include adapted viruses and kindness bacteria. Viral vectors are further competent than non - viral vectors for DNA delivery but may present a significant danger to patients, while non - viral carriers are naturally safer than viral carriers [Higashi et al.2009, Mastrobattista et al.2006]. Also, in contrast to the viral gene delivery systems, the non - viral carriers are usual to be less immunogenic, with simple preparation and a possible versatile outside alteration [Philippi et al.2010]. The non - viral vectors are usually made of lipids or polymers with/without using other inorganic materials where they can also be prepared from a lipid - polymer or lipid - polymer - inorganic hybrid. The choice of gene delivery strategies among several delivery systems depend on some factors including the improvement of vectors, kind of expression systems, and better understanding of molecular biology of target site and employing of the advances in the identification of new genes and new targets [Lu et al.2009]. Recently, nanotechnology approaches play an important role in the create novel and able non - viral gene delivery vectors. In this review, we will centre on introducing recently synthesized nanoparticles as vectors through gene delivery applications.

2. Delivery methods

Agrobacterium - Mediated Gene Transfer

Agrobacterium tumefaciens is a Gram - negative soil phytopathogenic bacterium which causes crown gall disease in plants and it can be grown in vitro in simple culture media with no any further plant growth hormones and the bacterium [Davey et al.2010]. This disease is recognized by neoplastic growth caused by the addition of the transferred DNA (T - DNA) obtained from specific DNA fragments (Ti) plasmid into the plant nuclear genome [Meyers et al.2010]. This characteristic is generally used in plant biotechnology, and Agrobacterium is the mainly significant tool employed to produce transgenic plants [Barampuram et al.2011]. In the past three decades, the biology of Agrobacterium and its associations with host plant have been the subject of a lot of studies, for latest review see [Braun et al.1942].

The accepting of the T - DNA combination pathways have very much enriched by releasing new studies and in the same time, it showing many host factors that participate in these events [Fraley et al.1985, Braun et al.1942]. T - DNA addition for Agrobacterium includes two major steps: initially, the T - strand is transformed to a double - stranded form; and secondly, the host cell DNA repair machinery mediates the double - stranded T - DNA mixing into double strand breaks in the host genome [Braun et al.1942].

This process has been found to be demanding due to the low duplicate number and large size of Ti plasmids, most important to problems in plasmid direction and separation, and it partial in the choice of plant species that can be altered since not all tissues or species are at risk to Agrobacterium [Tzfiraet al.2004, Stachelet al.1986]. To maintain these limits, it has been reported by the group of researchers in Japan and Australia Nakano, Suzuki [Merloet al.1980], Large DNA molecules about 100 kb incorporated by this indirect process and be likely to be rearranged with deletions, duplications and insertions not only in the

transgenic rice lines but also in itself. thus, this process may not be the perfect for conversion bulky DNA fragments. Next to a few drawbacks for Agrobacterium transformation, this means at rest works in numerous labs and high alteration rate frequencies could be obtained. More recently, by group of researchers Chetty, Ceballos [Gelvin et al.2000], four Agrobacterium tumefaciens strains (GV3101, EHA105, AGL1, and MP90) for the genetic revolution of tomato (Solanum lycopersicum L.) cultivar was evaluated. The reasons they chose Micro - Tom for their experiment was due to the fact that it differs from characteristic tomato cultivars by having two recessive genes, which present the dwarf phenotype and it is chosen as a model system for functional genomics, since it shares a number of unique features with Arabidopsis, such as minute size and short life cycle [Sone et al.2002]. Transformation rate frequencies for all strains were (65%, 40%, 35% and 15%) correspondingly. The analysis by comparative qPCR technique for duplex Taq - Man reactions showed that the strain MP90 was the mainly capable in the transport of single transgene insertions into the tomato genome apart from to their lowest transformation rate. Therefore, their discovery could be used for functional genomics and biotechnological applications into tomato.

Particle bombardment

Element bombardment is a hypothetically easy powerful tool for biotechnologists allowing direct gene transfer to a broad variety of cells and tissues which have been set up difficult to transform by other technique, and is more valuable for improving species which have high level of heterozygosity for instance potato and cassava [Gardner et al.1993].

Mainly, the atom bombardment device comprises of a mechanism to speed up the particles to required speeds and correct their saturation into the receiver cells. At first, the gun powder release device was used to go faster inert metal micro- projectiles covered with biologically active compounds [Sanford et al.1988]. Soon after, this device was replaced with inert gas helium (Biolistics1 PDS - 1000/He) to offer the force for microprojection [Kikkert et al.1993]. The come apart disk assemblage is the major part of this most commonly used device which controls the helium pressure and helium gas on the loose by a rupture disc and biased vacuum to propel a macrocarrier plastic sheet loaded with DNA coated tungsten or gold. This disk meeting contains a gas increase of rate tube with a break disk placed at the bottom of the tube inside a retaining cap. Afterwards, the gas chamber is displaced and the helium gas pressure is allowed to manufacture up to the desired level to break the rupture disk. The main function of the microprojectiles is to make holes through which DNA passes into the cells. Therefore, a number of modifications have been made to the power cause used to propel the microprojectiles to control microprojectile penetration into cells such as keeping up nitrogen gas pressure, an air gun and packed in air [Morikawa et al.1989, Iida et al.1990]. A most important difference in the air gun technique is that the DNA is undecided with the microprojectiles rather than being covered on them [Oard, et al.1990]. The creators of mentioned techniques supposed that the movement of DNA autonomous of microprojectiles which allows them to target tiny locations of tissue.

Viral vector systems

Recombinant viruses such as retrovirus, lentivirus, adenovirus, and adeno - associated virus have been broadly explored as vehicle vectors for therapeutic gene delivery [Walther et al. 2000]. In a lot of cases, these viruses are able to efficiently deliver genes by attractive cellular uptake through the utilization of intracellular trafficking processes. Large - scale production of the viral vectors, on the other hand, can be hard and they often also induce an acute inflammatory response with repeated dosing [Young et al.2001]. The potential addition of foreign DNA into the genome is also a major safety issue with viral vectors [Walther et al.2000].

Cationic polymers have also raised important interest for gene delivery. These polymers can be considered to form nanosized complexes, called polyplexes, when mixed with the anionic genes. These polyplexes are often more stable than lipoplexes [Al - Dosari et al.2009] formed with cationic lipids. Of the cationic polymers, polyethylenimine (PEI) is one of the more usually used and is measured one of the mainly effectual polymeric gene delivery materials [Al - Dosari et al.2009]. Available as branched or linear structures [Walther et al.2000], the gene delivery competence and cytotoxicity of PEI is primarily resolute by its size, molecular weight and the polymer: DNA charge ratio. For case, studies have shown that high molecular weight PEI (¤25 kDa) is more toxic to cells and is less useful at gene transport than polymers with medium to low molecular weight (5 - 25 kDa) [Fischer et al.1999].

Direct narrow injection of unmodified therapeutic genes has been practical intramuscularly [Wolff et al.1990] and in several other tissues for instance the liver, skin, and brain [Fischer et al.1999]. In some cases, this method may be good - looking to clinics because of its simplicity [Glasspool - Malone et al.1999]. Jet injection, which is needle free, is useful by driving gene solutions at high speeds using pressurized gas, usually CO2 [Wendell et al.2006]. The injection generates pores on target cell membranes and thus allows genes to enter into the cells. The power and depth of penetration can be controlled by modulating the gas pressure [Wendell et al.2006]. several of the side - effect's experiential with jet injections contain localized pain, edema, and bleeding at the injection site [Lysakowski et al.2003].

Hydrodynamic gene transfer

The hydrodynamic process uses improved liquid pressure as a forceful force for gene delivery. Studies in mice have second - hand a rapid, tail vein injection of a large volume of gene solution, typically 8 - 12% of body weight, in short time (3 - 5 sec), to deliver the target genes into the liver, lung, kidney, and heart [Liu et al.1999]. This system induces a reversible permeability change in the endothelial lining and generates pores in cell membranes to enable gene delivery [Zhang et al.2004]. The hydrodynamic method is moderately simple to apply and does not require time consuming preparations such as virus production [Liu et al.1999]. Large necessary doses and non - specific targeting, though, limit its clinical usefulness.

NPs for gene delivery

In the last only some years, lots of groups have reported the use of nanoparticles to complex and deliver viral vectors (e. g., adenoviruses, retroviruses) and nucleic acids, foremost to the appearance of new approaches known as magnetofection and the ranostics. Magnetofection is a viral and non - viral come up to that uses superparamagnetic nanoparticles to get better gene delivery under a magnetic field. The ranostics combines therapeutics with diagnostics and covers some fields, including personalized medicine, pharmacogenomics, and molecular imaging to develop efficient novel targeted therapies with an adequate risk/benefit ratio. Furthermore, the ranostics aims to monitor the reaction to treatment and to increase efficiency and safety.

Nanoparticles can be used to complex both nucleic acids (non - viral magnetofection) and viral vectors (viral magnetofection). Magnetic vector complexing involves the standard preparation of the gene vector prior to the mix between these vectors and the magnetic particles. Most published results reveal the need for additional agents to form the complex between the nanoparticles and the gene delivery vector. In the case of DNA, polymers can act as a link between the particles and the nucleic acid (Bryson et al., 2009; Kamau et al., 2006). Iron oxide nanoparticles (SPIOs) have been coated with transfection agents such as polyethyleneimine (PEI) and joined to DNA. When the mix is performed under a magnetic field, also permanent or pulsating, transfection competence increases 40 - fold over standard conditions (Kamau et al., 2006). Peng et al. (2016) demonstrated AuNPs for immediate gene and antimicrobial therapy by conjugating antimicrobial peptides with cationic AuNPs for gene delivery to mesenchymal stem cells. The typical methodology for the AuNP - based gene delivery is the functionalization on the surface of AuNPs with positively charged molecules, such as amino acids, cationic peptides, and molecules containing tertiary amines (Ye and Loh2013; Ye et al. 2015). AuNPs conjugated with oligonucleotides have proved their practical application in gene therapy (Mendes et al. 2017). Additionally, Shakil et al. (2019) successfully conducted IO NPs as theranostic agents for breast cancer gene therapy. It was demonstrated that the efficiency of DNA transfer increases by using a magnetic field leading to an increase in the delivery into the cellular compartments. A new study (Jin et al. 2019) proved that IO NPs could potentiate the gene silencing effect via targeting B - cell lymphoma - 2 (BCL2) in Ca9 - 22 oral cancer cells. Fascinatingly, SPIO NPs delivered siRNA against HIV-1 nef (anti-nef siRNA) into two cell lines, HEK293 and macrophage RAW 264.7 (Kamalzare et al. 2019).

Furthermore, Ohta et al. (2016) developed a nanocarrier classification based on single - walled CNTs designed of polycationic and amphiphilic peptides adapted by PEG. The cellular uptake of CNTs - peptide - PEG by A549 human lung adenocarcinoma epithelial cells showed the potential functional complex as an attractive candidate for anticancer activity. Also, Taghavi et al. (2016) fabricated single - walled CNTs loaded with PEG and polyethylenimine (PEI) modified by alkylcarboxylation to increase lipophilicity for vector delivery. Results verified that the nanocarrier could condense DNA into a size of 150 nm and improve the gene delivery of sh - RNA to MCF7 cells.

Fabrication for delivery

Chattopadhyay et al. (Chattopadhyay et al., 2006a, b; Shekunov et al., 2006), first successfully fabricated composite micro - and nanoparticles utilizing supercritical fluid extraction of emulsions (SFEE) for sustained - release drug formulations utilizing both batch and continuous processing. Model hydrophobic drugs such as indomethacin and ketoprofen were encapsulated in biodegradable PLGA and pH - independent swelling Eudragit RS polymers, forming composite particles ranging in size between 0.1 and 2.0 μm. The drug loading efficiency for composite particles was approximately 98% of the theoretical loading as determined by HPLC.

Amani et al. (2020) also synthesized a novel multi - targeted magnetic nanoparticle (MTMNPs) to investigate their potential application in DNA protection and targeted gene delivery to breast cancer cells. MTMNPs was prepared from polyethyleneimine coated Fe3O4 nanoparticles, polyethyleneimine - polylactic acidpolyethylene glycol - folic acid copolymer (PEI - PLA - PEG - FA) and DNA using double - emulsion solvent evaporation method. The EPPT peptide (Glu - Pro - Pro - Thr) was also incorporated into the MTMNPs as a targeting agent for overexpressed MUC - 1 receptors on breast cancer cells. The DNA release ratio from the MTMNPs nanoparticles were significantly decreased with increasing the pH value and decreasing the temperature.

Mayo et al. (2010) fabricated the PLGA 85: 15 polymeric matrix - type nanoparticles incorporating pFlt23K and pEGFP plasmid DNA using SFEE. Electron microscopy images indicated that the nanoparticles formed were discrete spherical particles. The SCF processing enabled plasmid incorporation efficiencies to reach > 98%, incorporate up to 19.7% w/w pDNA in PLGA, reduce residual organic solvent levels beneath detection, and release plasmid effectively from the nanoparticles.

Nucleic acids delivery mechanism in plant through NPs

RNA delivery mechanism using NPs

Although it is not a simple task to complete these goals, new advances in the field of nanotechnology have established that NMs are capable vectors with the capability to rise above a variety of barriers for successful RNA delivery. Now, we summarize five main NM - based delivery platforms, namely lipid, polymer, peptide, biomembrane, and inorganic NMs. Their advantages and disadvantages for RNA delivery are briefly shortened, and their current progress is discussed in detail lower. For those interested in RNA conjugates (e. g., GalNAc, lipid, and aptamer) and nucleic acid - based delivery method, we demote readers to reviews focused on these topics [Kaczmarek et al.2016, Desigaux et al.2007, Chen et al.2012].

Modern studies indicate a wide variety of NPs, including lipid and lipid derivatives, polymers, proteins, inorganic materials, and hybrid particles for the delivery of mRNA. These NPs explain favorable pharmacokinetics and potent pharmacological effects against multiple chronic diseases (Ramasamy et al. 2021). The composition and selection of NPs for mRNA therapeutics ensure stability and transfection

efficiency. mRNA triggers endosomal escape, prevents immune activation and provides clinically translatable NPs - based mRNA drugs.

Polymeric nanoparticles are a new class of nanoscale platforms for mRNA delivery. Formerly, a wide variety of polymers were designed and synthesized including polyamines, polypeptides, diblock polymers, and triblock polymers. In this section, we will describe representative polymers for mRNA delivery. Among these polymers, polyethylenimine (PEI) is a extensively used cationic polymer for nucleic acids delivery (Boussif et al., 1995). PEI isa group of linear or branched polyethylenimine polymers with tough affinity to nucleic acids and proton sponge effect for facilitating endosomal escape (Akinc, Thomas, Klibanov, &Langer, 2005; Demoulins et al., 2016). In 2016, Demoulins packaged saRNA encoding influenza virus hemagglutinin and nucleocapsid using linear or histidinylated PEI. These PEI/RNA nanoparticles were after that injected subcutaneously in mice, inducing equally humoral and cellular immune responses (Demoulins et al., 2016)

In accumulation to the platforms mentioned above, researchers also investigated other types of nanoparticles for mRNA delivery such as gold nanoparticles, polymer - lipid hybrid nanoparticles, and peptide complex. Gold nanoparticles (AuNP) thiolated with a short DNA oligonucleotide enabled balancing binding with definite sequences on mRNA (Chan et al., 2017; Chan, Chao, & Kah, 2018; Yeom et al., 2013). The hybrid region can be the 5′ - or 3′ - UTR, or poly (A) tail. Yeom et al. hybridized DNA oligonucleotide on AuNP with 5′ - UTR before the Kozak sequence of BAX (BCL - 2 - associated X - protein) mRNA. The AuNP/mRNA not only induced cell apoptosis in vitro but also considerably inhibited the growth of xenograft tumors in mice follow subcutaneous injection (Yeom et al., 2013). Lipids and lipid - like materials represent the second major class of nanoparticle - based delivery vehicles for RNA. As with polymers, cationic lipids are often used to electrostatically bind the nucleic acid. a lot of laboratories, though, have started utilizing ionizable lipids, which are lipids that are positively charged only at acidic pH. This ionizable behavior is thought to improve efficacy through helping with endosomal escape [Schroeder et al.2010]. As an alternative to nanoparticles, a more conceptually straightforward and chemically well - defined means of delivery is to directly conjugate a bioactive ligand to the RNA that will allow it to enter the cell of interest [Nair et al.2014].

Recently, Lee et al. (2015) incorporated polyarginine - fused heart - targeting peptide into Lipofectamine and showed an valuable delivery of mRNA, which induced direct cardiac reprogramming towards cardiomyocyte cells, as evidenced by increased expression of cardiomyocyte markers. In 2016, Kranz et al. (2016) explored the ratio of DOTMA/DOPE to mRNA and recognized an optimal ratio, 1.3: 2. Particle size of this lipid/mRNA complex was approximately 300 nm measured by Nicomp 380 ZLS. After injected intravenously, this complex showed systemic delivery into DCs and induced effector and memory T - cell responses. In this study, they also found that the ratio of lipid: RNA significantly varied surface charge, thereby leading to protein expression in different organs (Kranz et al., 2016).

3. Conclusion

Gene therapy has strained important thought as a possible method for treating equally sharp illnesses and chronic diseases. up to date research labours have purposeful on just beginning carriers that successfully solid and guard uncovered DNA, RNA and siRNA, which are fast tainted by enzymes in the blood. As an substitute to viral and polymeric carriers, nanoparticles have been introduced as shows potential carriers with low toxicity profiles and well - controlled gene delivery efficiency. While significant advances have been through for in vitro applications, much still remains to be done, mainly for in vivo change. At this time we supply a brief reconsider on the progress of nanoparticles for gene delivery.

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