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REVIEW ARTICLE

Liposome Based Nanoparticles for Gene Therapy: A Review

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Abstract:
There has been a growing significance of nanomaterials, particularly lipid-based nanoparticles (LNPs), as a promising alternative to conventional medication delivery methods. Over the past decade, LNPs have gained substantial attention in both preclinical and clinical research due to their remarkable pharmacological performance and potential therapeutic effects. This article specifically focuses on the application of nanoparticle-based liposome drug delivery systems (DDS) in the field of gene therapy. The study of these liposomal carriers for gene therapy is motivated by their ability to enhance the targeted delivery of therapeutic genes. The review discusses the rationale behind utilizing such a drug delivery system, emphasizing its potential to address existing challenges in gene therapy. Various aspects related to nanoparticle-based liposome DDS, such as formulation strategies, targeting mechanisms, and therapeutic efficacy, are thoroughly examined. Additionally, the article focusses on the current state of research and development in this domain, shedding light on recent advancements and emerging trends. The overall goal is to showcase the significant promise and potential of integrating nanotechnology with liposomal carriers for gene therapy applications.

Keywords: Nanomaterials; Lipid-based nanoparticles; Drug Delivery; Nanocarriers; Gene therapy

1. Introduction

According to J. Watson, who asserted, "We used to think that our fate was in our stars, but now we know, in large measures, that our fate is in our genes" [1], the potential of gene therapy in addressing genetic problems and offering significant therapeutic benefits cannot be overstated. As early as 1966, Tatum foresaw that mammalian cell transfection techniques would play a pivotal role in the future of medicine. Over the course of more than three decades of dedicated research, gene therapy has evolved into a viable and promising treatment strategy for a spectrum of human diseases. The fundamental organization of genes in cells from diverse origins has long been a focal point of cellular biology research. Beyond its utility as a research tool, gene transfer emerges as a revolutionary concept in gene therapy, representing a molecular therapeutic approach for addressing hereditary and other disorders [2]. The realm of hereditary diseases, encompassing conditions such as muscular dystrophy, cystic fibrosis, and familial hypercholesterolemia, has been a primary focus for gene therapy exploration [3]. The prospect of rectifying diseases rooted in genetic components through genetic refinement, entailing the incorporation of necessary genes, has become a central theme in gene therapy research.

However, addressing the challenge of correcting mutations and repairing genes presents a substantial hurdle. The sheer size of a gene, bound by multiple anionic charges, impedes its entry into a cell. To overcome this obstacle, various artificial procedures have been developed and employed for in vitro gene transfer [4]. Several strategies for gene transfer have been explored, including membrane disturbance through chemicals such as organic solvents and detergents, direct DNA microinjection, physical methods like mechanical or osmotic approaches and electric shocks, as well as the use of liposomes [5,6]. In the pursuit of gene therapy, various approaches are employed to correct faulty genes causing genetic disorders:

- **Replacement of Nonfunctional Genes:** Inserting a normal gene into a nonspecific site within the genome serves as a method to replace a nonfunctional gene.
- **Homologous Recombination:** This technique allows for the replacement of an aberrant gene with a normal counterpart.
- **Selective Reverse Mutation:** Addressing damaged genes can be achieved through selective reverse mutation.
- **Gene Regulation Adjustment (On/Off):** Fine-tuning a gene's regulation, toggling its on or off status, is another avenue explored in gene therapy.

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The main objective of this study is to comprehensively explore the potential and challenges of nanoparticle-based liposome drug delivery systems in gene therapy, emphasizing their role in enhancing targeted delivery, overcoming existing obstacles, and highlighting recent advancements and emerging trends in the field

2. Vectors for gene therapy

Two techniques, ex-vivo and in-vivo, have been employed for delivering vectors in gene therapy. In the ex-vivo method, cells are extracted from patients, normal genes are cloned into the vector, and then combined with cells containing faulty genes. The transfected cells are reintroduced into the patient to produce the required protein for treating the illness. Conversely, the in-vivo method involves introducing vectors with normal genes directly into the patient's bloodstream to interact with target cells. [7]

Gene therapy utilizes various vectors, including viral vectors like retroviruses, which were the initial vectors in trials. Retroviruses use RNA as their genetic material and integrate their genome into the host cells' chromosomes, allowing modified host cells to pass on altered genes to their progeny. Adenoviruses, with double-stranded DNA, do not integrate their genetic information into the host cell but may trigger immune responses. Adeno-associated viruses (AAVs), despite their small size, infect both dividing and non-dividing cells accurately on chromosome 19, presenting a potential vector with limited payload. Herpes simplex virus (HSV) primarily transfers genes in the nervous system and allows for introducing multiple therapeutic genes due to its extensive genome. [8]

Non-viral methods, including direct DNA injection and various techniques such as electroporation, sonoporation, magnetofection, gene guns, and receptor-mediated gene transfer, offer alternative strategies for gene transfer, each with its own set of advantages and drawbacks. Clinical experiments with non-viral methods, such as injecting naked DNA plasmids, have shown effectiveness, while ongoing research explores the potential of these diverse methods in gene therapy. [9]

3. Liposomes for Gene therapy

Targeted liposome gene therapy exhibits therapeutic potential through diverse targeting mechanisms. However, in vivo systems have primarily delivered reporter genes. Upon systemic injection, the efficacy of targeted liposomes is enhanced through receptor-mediated endocytosis. This process facilitates the penetration of targeted liposomes into cells before undergoing lysosomal breakdown. The incorporation of PEGylation into lipids within liposomes aims to enhance their circulation survival, yet this approach has been associated with increased endosomal escape, consequently diminishing delivery efficiency. Strategies to address PEG-induced endosomal escape include making it cleavable, utilizing multiple ligands, or creating enzyme-triggerable versions. Recent advancements in targeted ligand design have contributed to the development of effective liposomal delivery systems, complemented by modifications to the PEG moiety that enhance serum stability [10]. Liposomes, minute vesicles formed by the self-assembly of phospholipid molecules, possess a structure akin to biological cell membranes. Comprising hydrophilic heads and hydrophobic tails, phospholipids self-assemble into a bilayer membrane structure in water. In an aqueous solution, the hydrophobic tails avoid water contact, while the hydrophilic heads face outward, resulting in the creation of a liposome structure resembling biological cell membranes [11].

Liposomes are categorized based on size, the number of phospholipid bilayers, synthesis method, and manufacturing technique. Three size classifications exist: tiny, medium, and giant. The number of membrane layers determines whether they are unilamellar (ULVs), oligolamellar (OLVs), or multilamellar (MLVs). ULVs, characterized by a single phospholipid bilayer measuring 50-250 nm, differ from larger MLVs (0.5-1.5 μm), which encompass multiple phospholipid bilayer membranes [12]

4. Liposome Based Nanoparticles (LNPs)

4.1. Characteristics

4. Liposome Based Nanoparticles (LNPs) have evolved from spherical particles to flat ellipsoidal shapes with diameters of 50-100 nm, offering increased stability compared to liposomes. LNPs can incorporate active materials in the lipid core or shell, enhancing particle stability. The addition of cationic lipids improves LNPs nternalization, aiding in better tumor targeting, blood-brain barrier penetration, and gene transfection efficiency. Macromolecules like oligosaccharides, proteins, ligands, and antibodies can modify LNPs ' outer shell, enhancing treatment site specificity [13].

4.2. Rationale for selection of LNPs

The rationale for choosing liposomes in nanoparticles-based formulations lies in their self-assembly mechanism, forming spherical vesicles with lipid bilayers that can easily entrap hydrophobic drug molecules. Liposomes, being amphiphilic, have hydrophobic

tails and hydrophilic head groups, creating stable lipid bilayers. They can entrap drugs through passive or active loading methods, offering versatility in size and shape. Liposomes have been recognized for gene and DNA delivery, overcoming challenges such as plasma nuclease sensitivity and intracellular barriers. While gene-based therapies face obstacles, liposomes show promise in encapsulating gene therapies, emphasizing the need for integrative agents for successful intracellular delivery, particularly in systemic gene delivery systems targeting specific cells [14]

4.3. Preparation

Nanoparticle synthesis is typically accomplished through two main approaches: bottom-up and top-down. The bottom-up method involves the transformation of atomic-sized materials into nanoparticles, employing techniques such as gas phase synthesis, block copolymer synthesis, Turkeyvich method, and microbial synthesis. On the other hand, the top-down strategy involves physically breaking down bulk materials into nanosized particles through processes like milling, spark ablation, and laser ablation [15].

Due to their distinctive colloidal structure, (LNPs are commonly synthesized using wet chemistry through the bottom-up approach. This section explores various LNP synthesis techniques, including nanoprecipitation, single/double emulsification, nonsolvent emulsification, thin film hydration, microfluidic processes, and impingement jet mixing technology [16].

The choice of LNP synthesis method is crucial for therapeutic applications, impacting their physicochemical characteristics, drug loading efficiency, stability, and in vivo behavior. Each LNP production technique generates nanoparticles with unique properties that influence their effectiveness in therapeutic applications [17]. Various methods employed for LNPs are:

- Nanoprecipitation
- Single/Double Emulsification
- Nonsolvent Emulsification
- Thin Film Hydration
- Microfluidic Processes
    - T- or Y-Mixer
    - Hydrodynamic Flow Focusing
    - Staggered Herringbone Micromixer
    - Bifurcating Mixer
- Impingement Jet Mixer
- Scaling-Up LNP Production by Microfluidic Devices

4.4. Targeting mechanisms

Focused on providing high-quality treatment, liposomes show clinical promise due to their ability to target various substances. However, in in vivo systems, the reliance on correspondent genes has been a common approach. Receptor-mediated endocytosis facilitates the entry of targeted liposomes into cells before lysosomal degradation after systemic administration. PEGylation of lipids has been utilized to improve circulation survival, but the presence of PEG is associated with increased endosomal leakage, reducing delivery efficiency in all examined *in vivo* studies. [18]

Efforts to address PEG-induced endosomal leakage include making PEG cleavable, using double ligands, or creating enzyme-induced mutants. Despite these strategies, many liposome complexes, coupled with targeting ligands, fail to reach the desired target site, often accumulating in the liver due to low interaction between target and targeted liposomes. [19]

Transmission effectiveness is limited, and targeting is highly cell-dependent due to different surface ligands on various cell types. The development of efficient clinical systems for delivering nucleic acid therapeutics depends on factors such as ligand design, target cells/tissues, liposome composition, and liposome-cargo interaction. In vitro studies have predominantly focused on various cancer cell lines, but recent investigations are starting to consider cell-type-specific approaches. Although targeting ligands like RGD peptides can specifically target tumors, obstacles such as abnormal blood flow through tumor blood vessels impede effective cargo penetration into tumor tissue. Antibody-based targeting of cancer cells, particularly MCF-7, exhibits high expression efficiencies compared to peptide-based targeting. Recent studies suggest that combining new cationic lipids, such as DDCTMA and MSO9, with folate targeting can enhance efficiency. [20]

Efforts to identify efficient clinical systems are ongoing, with many preliminary studies focusing on providing reporter genes with limited therapeutic impact. Recent in vivo studies have preferred antibody- and aptamer-based targeting in mouse models for systemic administration to control tumors. Evaluation of target tissue toxicity is crucial in testing procedures, considering the heterogeneity of cell populations and changes in vascular organization associated with tumor growth. Overcoming the

pharmacokinetic challenges of liposome-nucleic acid systems by increasing cargo dosage may lead to dose-limiting toxicity, emphasizing the need for routine assessment of target tissue toxicity. [21]

4.5. Ligand Conjugated liposomes

Peptides offer attractive qualities as targeting ligands, such as small size, easy preparation, and high stability. Various peptides target integrin receptors, growth factor receptors, and G protein-coupled receptors. Peptide amphiphile derivatives, synthesized through solid-phase peptide synthesis or thiol-maleimide coupling, are commonly used for liposomal gene delivery. Notably, RGD peptides are frequently employed both in vitro and in vivo, although issues like uptake by the reticuloendothelial system exist. [22]

Folic acid/folates serve as essential ligands due to ease of conjugation with liposomes and overexpression of folate receptors in certain cells. Folate-bound liposomes enhance colloidal stability compared to peptides but may exhibit nonspecific interaction with folic acid carriers on normal cells. Limited in vivo examples of liposomal gene therapy targeting folate receptors exist, with FRα being a promising candidate for ovarian cancer treatment. [23]

Aptamers, short DNA/RNA oligonucleotides created by SELEX, have emerged as promising ligands. They offer advantages over antibodies, including ease of production, higher target antigen recognition, and non-immunogenicity. Aptamer-functionalized liposomes have been developed for nucleic acid delivery, with a focus on specific receptors like the transferrin receptor (TfR) and nucleolin. Aptamers show potential for delivering therapeutics to tumor microenvironments and overcoming immunosuppression, with ongoing efforts to address stability issues in targeted delivery systems. [14]

5. Future directions

Since the FDA approval of the first nanodrug delivery system, Doxil®, in 1994, liposomes have gained prominence in drug delivery. While they reduce toxicity and enhance breast cancer treatment, overall effectiveness remains a concern due to side effects like 'hair loss.' Liposomes meet key criteria for delivery vehicles, offering biodegradability, biocompatibility, and stability. Despite successful use as drug carriers, establishing a liposome formulation for gene delivery in breast cancer treatment remains unachieved. Cationic liposomes are favored, allowing easy modifications for virus-free gene carriers, while neutral liposomes show promise with versatile modifications. The combination strategies demonstrate the adaptability of liposome formulations for breast cancer therapy. [1, 15]

However, crafting the optimal liposome formulation with the correct lipid combination proves challenging, especially for functional nonviral gene carriers with extensive surface modifications. Over 4,444 liposome-based nonviral gene carriers are in the design phase, anticipating future preclinical development for cancer treatment. Successful translation necessitates targeting specific ligands of overexpressed receptors at metastatic sites, requiring optimization of treatment systems for in vivo adaptability. Despite challenges, liposomes hold potential to overcome clinical barriers and transform cancer treatment, reducing suffering associated with traditional approaches. Ongoing efforts are crucial to surmount physiological barriers hindering liposomal gene delivery systems in breast cancer treatment. Liposome-based gene therapy strategies are poised to drive breakthroughs in personalized medicine, fostering collaboration across diverse fields for innovative cancer treatments and other genetically related diseases. [2, 6]

6. Conclusion

In conclusion, the pursuit of targeted liposome-based gene delivery systems presents both significant advancements and persistent challenges, with a focus on the intricate interplay between ligand-receptor interactions, liposome stability, and the complex biological milieu. While in vitro studies predominate, notable in vivo successes, particularly in oncology, underscore the therapeutic potential. Antibody- and aptamer-based ligands show promise, expanding the horizons of targeted gene therapy. The quest for an ideal liposomal vector with low toxicity, high stability, and optimal characteristics remains crucial, requiring attention to manufacturing considerations and scalability for practical clinical applications. Looking ahead, the integration of dual-targeting systems and combination treatments holds promise for novel therapeutic strategies, marking a new era in precision medicine.

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Author's short biography

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Vrushti Shah is currently pursuing her last year of B Pharm. She has developed keen interest in latest technology and pharmaceutical research.

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Hello, I'm Priyanshu Gaekwad a Student of Pharmacy with a passion for Research and Analysis also interested in Virology and Epidemiology. With a bachelors in Pharmacy, I have cultivated a strong foundation in Pharmaceutical Trend Analysis and Microbiology. I am currently completing my Bachelor's Degree from ITM(SLS) Baroda University, Vadodara, Gujarat, India.