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Liposome Mediated Transfection in Eukaryoic Cell: An Overview

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Abstract
In Gene therapy Nucleic acids can be inserted into human cells to provide enhancement or reduction of protein expression for the prevention, treatment or elimination of the problem. For this to happen foreign gene should be inserted in to the cells using different mechanisms. The commonly used ways of gene delivery in to eukaryotic cells are of viral and non viral delivery systems. The non viral delivery are of different types of which the liposome mediated tranfection is the most widely used. Liposome mediated transfect ion represent approved non-toxic biocompatible nanoparticles for the application of medicine. Excellent biocompatibility, low immunogenicity, delivery of large piece of nucleic acid and simplicity of handling are the major advantages one has to see in liposome transfection. But the presence of positive charge on liposomes vectors which may favour nonspecific interaction with negatively charged serum protein, enzymes and causing in to decreased cell adhesion, hemolysis and low transfection are among the disadvantages. In this short review different types of liposomes and their reagents are highlighted. Generally the use liposome in the area of biomedicine (not only for gene delivery) gives a paramount relief specially in this era of drug resistance, vaccine failure, threatening emerging and re-emerging pandemics in the globe.
Keywords:-Liposome, Gene therapy, Transfection, Eukaryotic cell

1. INTRODUCTION TO GENE THERAPY

Nucleic acids can be used as a drug for diseases of known genetic basis by correcting missing genes, replacing defective genes, or down-regulating aberrant gene expressions with efficient delivery. There are mainly two classes of vehicles for gene delivery: (i) viral and (ii) non-viral vectors[2]. In Gene therapy genetic material is inserted into human cells to provide enhancement or reduction of protein expression for the prevention, treatment or elimination of disease. However, clinical implementation has been difficult[4].

Gene therapy starts with the identification of mutant gene causing the disease, cloning the identical healthy gene (therapeutic gene or transgene) which suppress or repair the problem. After producing the therapeutic gene it is loaded in a vector vehicle (the most critical step) so that to deliver the therapeutic gene to the patient target cell. Then the genetic material is delivered to the nucleus and integrated into DNA and corrects the defective or mutated gene(figure1)[5]. Gene delivery by using Lipid is most successful in both dividing and non-dividing cells where DNA entrance into the nucleus but entry to later cells happens at a low frequency[6].

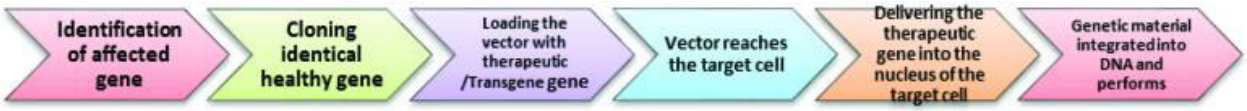

Figure 1: schematic illustration of gene therapy[5]

Adverse immunogenic reactions, insertional mutagenesis, and toxicity limits the application as well as the use of clinical trials in viral vectors where as Non-viral vector which uses positively charged polymers, peptides, or lipids forms self-assemblies with high DNA carrying capacity, ease of preparation, and lower immunogenicity and cytotoxicity but less efficient as compared to viral vectors[2].

2. PRINCIPLE OF LIPOSOME MEDIATED GENE DELIVERY

Liposomes are among non-viral vectors formed from hydrophilic head group and hydrophobic tails and then self-assemblein a dissolved lipid molecules. The energetically favorable interacting lipids forms a vesicular fluid entities with versatile supramolecular assemblies which can be used for gene and drug delivery as a liposomes[7].
Liposomal drug delivery systems are industrial technologies which represent approved non-toxic biocompatible nanoparticles for the application of medicine. They are used for the entrapment of lipophilic and hydrophilic agents likedrugs into the liposomes to bypass the frequent toxicity or inappropriate drug formulation.The formulation of the Liposomeof desired composition, morphology, size distribution and surface modification depends on cells that is going to be transfected[8].

The non-viral vector gene delivery is influenced by the quantity and quality of deoxyribonucleic acid (DNA), ratio of DNA to material, serum, antibiotics, solvent for DNA and material, incubation time, and mixing order. Lipofectamine™ 2000 (Lipo2000) is one of the highly efficient and most widely used commercial cationic lipids for in vitro gene transfer. The unbinding of DNA from CLs is not completely understood, but thought to result from charge neutralization by cellular anionic lipids that changes structure of lipoplexes

radically upon interaction with cellular lipids taken up by a cell via the plasma membrane, undergo endosomal escape in order to avoid lysosomal degradation and active transport to the nucleus for subsequent transcription (figure 1)[9].

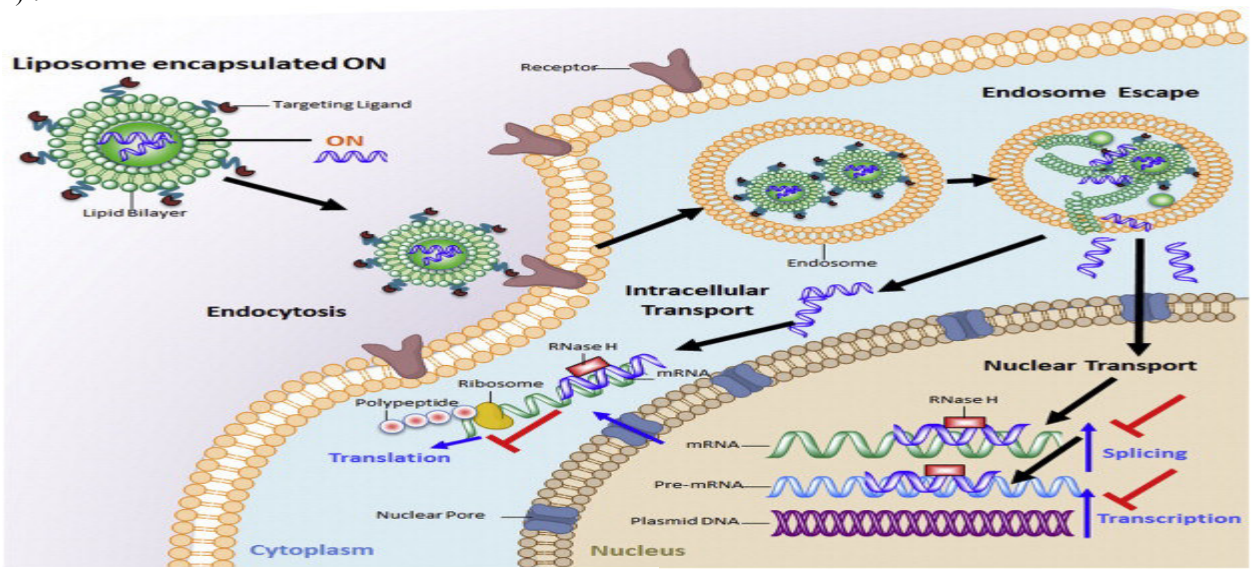

Figure 2. Liposome-mediated transfection and endocytosis[10].

To avoid these mentioned disadvantages heterocyclic ring with positivechargesare used[11]. But all this exhaustive and extensive efforts yield very limited improvements clinically[12] .

Due to variability of morphologies, compositions, abilities to envelope and protect many types of therapeutic biomolecule as well as lack of immunogenic response, low cost, and their differential release characteristicsthe applications of lipid vesicles is also variable[7].

The synthetic liposomes spheres withone or more bilayered membrane surrounding an aqueous core to encapsulate small molecules are used (**Figure 3A,3B**). Self-assembly of liposome happen due to the interaction of lipids with DNAand influenced by preparation procedure, mixing ratio, DNA concentration, and size of the cationic liposomes and ionic strength of the buffer[11].The transfection efficiency improvement and cytotoxicity reduction forced synthetic modifications of the liposome by using positivecharges ofhead group. This is done by introducing heterocyclic ring (imidazolium pyridinium) and protonated polyamine groups into cationic liposomes to decrease the positive charge of the cationic head as well as modification with the linker functionalization group which improve the properties of liposomes.Ether linked liposome arenot biodegradedand have higher toxicity despite their good transfection efficiency[13], but ester or amide linkers are more biodegradable and less toxic in cultured cells and are alsodegradable in the circulatory system[14]. Besides, polyethylene glycol (PEG) and/or other molecules such as ligands and peptides linked cationic liposomes (**Figure 3C,3D**) has been shown as a great improvement leading to small particle sizes, controlled structures, regular morphology, and good stability[15]. A number of structures appeared during polynucleotides compaction into liposomal assemblies. A specific structure of the assembly happen with energetically favorable conformation.(figure3)[16].

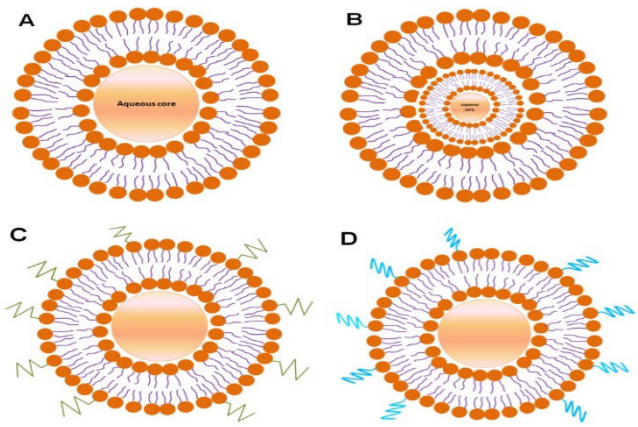

Figure 3: Schematic representation of the structure of liposomes(Adapted from[16]).
A: One bilayered membrane liposome
B: Two bilayered membrane liposome
C, D: linked cationic liposomes

3. TYPES OF LIPOSOME

3.1. Cationic lipids

The most commonly used commercial reagents for cationic lipid transfection are N-[1-(2,3 dioleyloxy)propyl]-N,N,N-tri methyl ammonium chloride (DOTMA),[1,2-bis(oleoyloxy)-3-(tri-methyl ammonio) propane] (DOTAP), 3β[N-(N′, N′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol)], and dioctadecylamidoglycylspermine (DOGS). Dioleoylphosphatidylethanolamine (DOPE is a neutral lipid used in together with cationic lipids infavour of its membrane destabilizing effects in endolysosomal escape[16].

Cations together withpolymers, lipids, and non-degradable nanoparticles theyare used asnon-viral gene deliver vectors.Hydrocarbon of both symmetric and asymmetric chains are used in a lipid mixture, but asymmetric lipid with both shorter saturated and long unsaturated carbon chains have a relatively high transfection efficiencies compared to mixed symmetric formulations of cationic lipids. Hydrophobic tails and ionizable head groups of multivalent cationic lipids (DOSPA and DOGS)serve as a buffer during protons influx into a maturing endosome/endolysosome.This is the reason why multivalent cationic lipids have higher transfection efficiencies compared to their monovalent counterparts[17].

3.1.1. Monovalent Cationic Lipids

A. DOTMA

N-[1-(2, 3-dioleyloxy) propyl]-N, N, N-trimethylammonium chloride, or DOTMA, is the first to be synthesized and commercially available cationic lipids in gene delivery. The structure consists of 2 unsaturated oleoyl chains (C18: Δ^9), connected by an ether bond to three-carbon skeleton of glycerol and quaternary amine as the cationic head group**.** DOTMA interacts spontaneously with DNA to form lipid-DNA complexes.This lipid facilitates fusion of the complex with the plasma membrane of tissue culture cells which leads to uptake and expression of the DNA. It is a simple transfection methodwith high reproducible capacity applied for both transient and stable expression of DNA at about100-fold more effective than either the calcium phosphate or the DEAE-dextran transfection[18].Transfection efficiencies was increased by using Combination of DOTMAwith DOPE inan equal ratio which adds functional groups to the main lipids. Manipulation in the head group, linker, linkage bonds, and hydrocarbon chains of DOTMAhave indicated reduction in toxicity and increased transfection efficiencies[19].

B. DOTAP

Leventis and Silvius in 1990 synthesized [1, 2-bis (oleoyloxy)-3-(trimethylammonio) propane], or DOTAP for the first time. The molecular sturucture consists of a four amine head group connected to glycerol backbone with two oleoyl chain linked by ester bond. This monovalent lipids also showed almost no cytotoxic effect on cell monolayers with good lipoplex sensitivity at 25%–35% cell confluency[19].The use of naked DOTAP is inefficient due to its protonation at pH 7.4.This costsmore energyto separate the DNA from the lipoplex for efficient transfection. Thus, DOTAP should be combined with a helper lipid like most cationic lipid formulations[7].

In both DOTMA and DOTAP resistance to serum interaction is observed even though the way how the interaction happens is unknown but it ishypothesized that binding to cell membranes, structural complex maturation inhibition, binding to cationic charges and the disparity of endocytosis pathways are the speculation.[20].

C. DC-Chol

Gao and Huang in 1991 synthesized for the first time [3β [N-(N', N'-dimethylaminoethanecarbamoyl] cholesterol, or DC-Chol by attachingcholesterol moiety using an ester bond to the main structure. DC-Chol waschosen for its biocompatibility, stability to lipid membranes and have a four timesreduction in cytotoxicity compared to Lipofectin in some cell lines[21]. DC-Chol, in equal lipid ratio with DOPE, contains a tertiary amine where 50% of the liposome surface is charged at pH 7.4 as opposed to DOTMA and DOTAPwhich are fully charged[22]. This charge reduction helps in DNA dissociation, successful transfection and higher transgene expression[23].

3.1.2. Polyvalent Cationic Lipids

A. DOSPA

DOSPA is structurallyanalogues to DOTMA but the spermine group here isconnected to hydrophobic chainsby peptide bond. The commercial available transfection reagent Lipofectamine is formed of this cationic lipid and DOPE at a 3: 1 ratio.Efficient packing of DNA is achieved by the addition of the spermine ammonium functional group[7].

B. DOGS

Structurally Di-octadecyl-amido-glycyl-spermine, or DOGS is similar with DOSPAby the presence of polyvalent spermine head group and alkyl chains of the two 18-carbon. Clear differences are observed on the chains of DOGS which are saturated and linked to head group by a peptide bond on top lacks a quaternary amine group. This kit is commercially available as Transfectam to be used in many cell lines with good efficiency up to 10-fold better than calcium phosphate transfections with no noticeable cytotoxicity[24].

Additional to efficient condensation of DNA, the delivered DNA from degradation wasprotectedby head group of DOGS buffering ability of the endosomal compartment. DOGS do have buffering capacityat pH

values below 4.6 whenall of the amino groups in the spermine are protonated, but at pH = 8 only two are ionized, and promotes arrangement into a lamellar structure[25].

3.2.Modified Liposome

3.2.1. Poly (ethylene) Glycol

PEG is a linear polyether diolthat modifies physical adsorption onto the liposomal surface and its covalent attachment onto premade liposomes. This results in less toxic, biocompatible, ready excretion and ease of application. The presence of PEG on the surface of the liposomal carrier has been shown to extend blood-circulation time while reducing mononuclear phagocyte system uptake (stealth liposomes) of the intended gene to be delivered[26].

The PEG-coating has sterically stabilizes the liposomal membrane against interactions with destabilizing and opsonic factors in vivo. As a result it shows longer circulation times and reduced uptake by the Macrophages, relative to conventional liposomes. Although the exact mechanism behind the Macrophages avoidance phenomenon assumed that by the formation of a highly hydrated shield of polymer molecules around the liposome which sterically inhibits both electrostatic and hydrophobic interactions of serum components with the liposomal bilayer[1].

3.3. Neutral Lipids

A. DOPE and DOPC

Inclusion of “helper” lipids in the cationic liposomes improves lipoplex efficiency by increasing lipid self-assembly (micellar, lamellar, hexagonal, vesicular, etc.), the level of hydration, and DNA secondary and tertiary structure. 1, 2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), is used as a helper lipid for in vitro transfection with bettermatching of charge density to the DNAequal ratio of DOTAP/DOPE is neutralized and complexed with the negatively charged DNA. Due to salt bridges of the positively charged head groups of the lipids and the phosphate groups of DOPE. This would force the stabilization of primary amine group of DOPE to itself by allowing more close interactions with the negatively charged phosphate of the DNA. This reducespositively charged lipid head group, thus lowering the energy required for binding DNA[23].

DOPC is a much better helper lipid for in vivo transfection[27]. There are also Galactosylated cholesterol derivatives with decreased cytotoxicity and improved transfection efficiencies in Hep G2 cell lines. This is probably due to thehigh affinity of cellular receptors for galactosylated ligands for specific uptake[28].

3.4. Anionic Liposome

The most commonly used anionic lipids are phospholipids which are found in cellular membranes naturally like phosphatidic acid, phosphatidylglycerol, and phosphatidylserine. This lipids also have fatty acid chains in the hydrophobic region. Even though they are not efficient gene delivery agents because of negatively charged head group that prevents efficient DNA compaction as a result of electrostatic repulsiveforces the phosphate backbones of DNA they are selected because of resolving cationic lipids draw backs. Among which serum inactivation, unstability upon storage, and *in vitro* and *in vivo* cytotoxicity aresolved by these group of lipids. Here DNA-containing liposomes are formed by divalent cations bydenying the mutual electrostatic repulsion to form lipoplex assembly. The incorporated fatty acids are responsible for the fluidic characteristics in terms of phase behavior and elasticity[29].

3.5. pH sensitive liposomes

These liposomes are destabilized by the endocytotic pathway because of the effectof acidic conditions and pH-sensitive lipid components. This enables thecontent to be delivered into the intracellular bio-environment or its fusion with the endosomal membrane. Their therapeutic efficacy allow them to be used as biomaterial with commercial utility especially in cancer treatment. Another way how this liposome, are used is by targeting ligands (antibodies) which can be anchored on the surface ofpH-sensitive liposomes so that they targets specific cell surface receptors/antigen present on tumor cells[30].

3.6. Fusogenic liposome

The discovery of Okada et al 1985 describes conventional liposome as well as cell membranes can efficiently be fused by Sendai virus using virus envelope protein to form fusogenic liposomes. Commercially it can be prepared when unilamellar liposomes are fused with the purified Sendai virus. Fusogenic liposomes delivered their contents into the cytoplasm via an endocytosis-independent pathway as compared to conventional liposomes which are taken up by endocytosis. The main advantage of Fusogenic liposomes is to avoid the immediate split of the gene by lysosomal enzymes, thus; providing a vehicle for the delivery of the contents into the cytoplasm (figure 4). These liposomes can also be used to deliver encapsulated contents into the cytoplasm of a wide variety of mammalian cells[3].

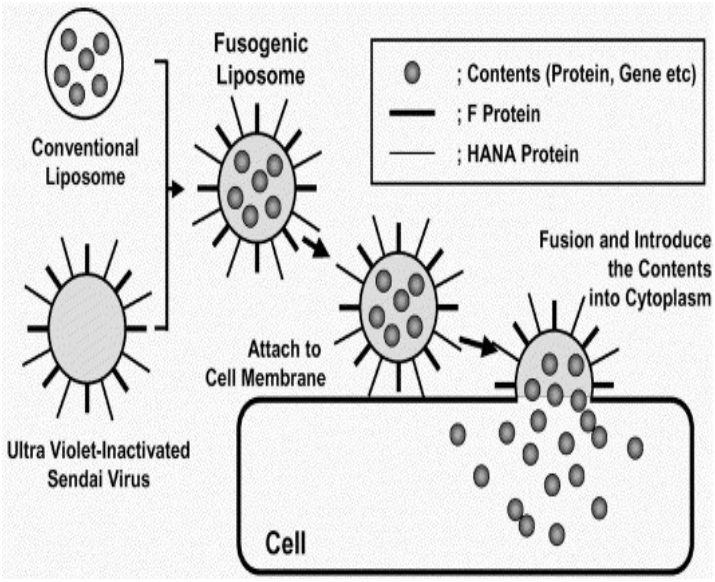

Figure4: Fusogenic liposomes as efficient delivery vehicles into the cytoplasm[3]

3.7. Immunoliposome

This was first described as antibody-targeted liposomes (immunoliposomes) by Torchilin et al in early 1970eswhere it specifically bind to the antigen that is expressed on the target cells. After wards coupling techniques were used for conjugating antibodies or their fragments to liposomes (Figure5). Up on in vitro experiments immunoliposomes are highly specific in binding to target cells. Sterically stabilized PEG–liposomesare used for coupling antibodies by different methods. The coupling is done by attaching the ligand directly to the liposome bilayer and attaching the ligand to the terminal end of PEG. The clearance rate of PEG–immunoliposomes is dependent on the antibody density at the liposome surface. At low antibody density (<≈50 μg mAb/μmol PL), the PEG–immunoliposomes are cleared at rates slightly more rapidly than antibody-free PEG–liposomes but very rapid at higher antibody density (>100 μg mAb/μmol PL)[1].

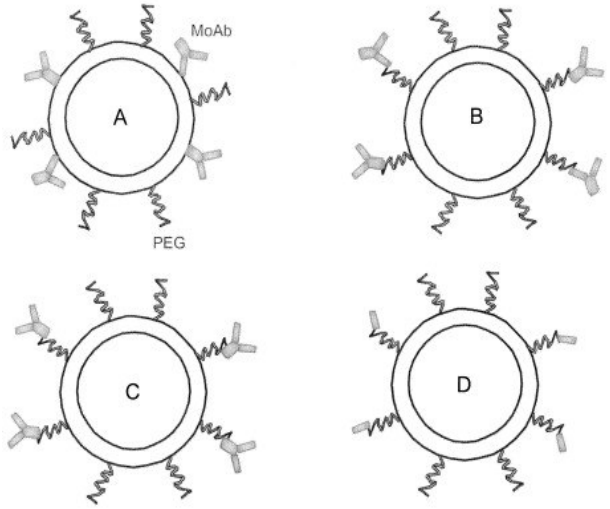

Figure5: Currently used PEG–immunoliposomes types[1]

4. CONCLUSIONS

Nucleic acids is a drug for diseases in that it helps correcting genetic problems and protects from infectious diseases. For this to happen an efficient delivery system should be chosen. Known delivery system are of the viral and the non-viral ones with both having their own advantage and disadvantages. But the non-viral delivery reagents are better than the viral delivery methods in that the disadvantages are less as compared to the viral or modification may change the behavior of the agent. Among the non-viral vectors liposomes having various array of morphologies, compositions, capabilities to envelope and shield many types of therapeutic biomolecule are the best transfection reagents. So the use of these liposome in the area of biomedicine gives a paramount relief specially in this era of drug resistance, vaccine failure, threatening emerging and re-emerging pandemics in the globe.

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