Novel Application of Nanotechnology in Drug and Gene Delivery: Emphasis on Liposomes

Esmaeil Nourmohammadi[1], Reza Nedaeinia[2], Mohammad Goli[3], Saeed Hosseini Teshnizi[4], Hoda Khoshdel Sarkarizi[5], Mozhdeh Nedaeinia[6], Habibollah Faraji[7]*

[1] Students Research Committee, Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Deputy of Food and Drug, Isfahan University of Medical Sciences, Isfahan, Iran
[3]Department of Food Science and Technology, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
[4]Department of Biostatistics, School of Medicine, Shiraz University of Medical Science, Shiraz, Iran
[5]Department of Anatomical Sciences and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[6] Young Researchers and Elite Club, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran
[7]Department of Laboratory Sciences, Faculty of Para-medicine, Hormozgan University of Medical Science, Bandar Abbas, Iran.

ABSTRACT

Nanotechnology is a branch of science which has engineered and manufactured materials at the atomic, molecular, and supramolecular scale. Nanotechnology refers to structures at roughly the nano-metric scale (10^{-9} m) in at least one dimension. Here, we focus on the application of nanotechnology to drug delivery, with emphasis on Liposomes. Several applications could enable entirely novel classes of therapeutics. The production of various systems of liposomes at nano scale and the integration of the resulting nanostructures into larger systems is interesting. In this regard, the effect of nanotechnology in early diagnosis and targeted drug therapy of diseases such as cancers, diabetes, infections, etc. is indubitable. Indeed, the small size, customized surface, improved solubility, and multi-functionality of nanostructures can lead to better bio-compatibility, bio-accessibility, and efficacy under different conditions.

Key Words: Nanotechnology, Drug Delivery, Gene delivery, Liposomes.

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INTRODUCTION

Treatment of diabetes has a number of challenges in terms of the route of delivery and inadequate glycemic control [1]. Availability of insulin in non-parenteral dosage form will be a breakthrough and development of an appropriate drug-delivery device can contribute to this approach [2]. Chemotherapy drugs for cancer also have poor cell-specificity and high toxicity, leading to bone marrow suppression, gastric erosion, hair loss, renal toxicity, cardiomyopathy, and many such impacts on other systems [1]. Hence, scientists are developing a wide spectrum of nano-scale technologies for changing the scientific landscape with respect to diagnosis, treatment, and prevention of different diseases. Nanotechnology includes the production and application of physical, chemical, and biological systems at scales ranging from individual atoms or molecules to around 100 nanometers, as well as the integration of the resulting nanostructures into larger systems [3]. The combination of bioactive nanomaterials with a biomaterial scaffold shows promise for the development of a localized long-term treatment [4]. Biodegradable polymers have been studied extensively over the past few decades for building these drug-delivery vehicles. These nanoparticles could mimic or change biological processes (e.g. infection, tissue engineering, de novo synthesis, etc.) [5].

Corresponding author: Habibollah Faraji
Address: Department of Laboratory Sciences, Faculty of Para-medicine, Hormozgan University of Medical Science, Bandar Abbas, Iran.
E-mail: ✉ Farajih901 @ hums.ac.ir
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This article presents a review of nanotechnology for the biologist and discusses novel methods in delivering drugs and genes using liposomes, as well as delivering genes using some other nano particles.

Liposomes and drug delivery

Liposomes—a part of drug-delivery systems at the nano scale—were discovered in mid-1960s. These spherical nanoparticles with lipid bilayer membranes have two components—aqueous interior for water-soluble drugs and lamella of membrane (unilamellar or multilamellar) for lipid-soluble drugs [2] (Fig 1).

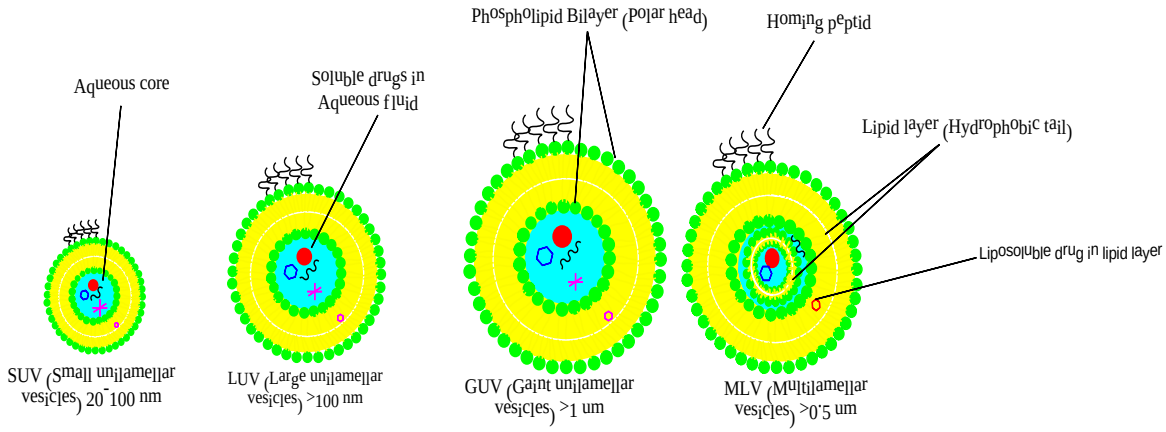

Figure 1: Comparison of different types of liposomes and their diameters.

Cancer chemotherapeutic drugs and toxic drugs like amphotericin and hamycin are more efficiently and safely delivered by liposome [2]. The stability of basic fibroblast growth factor (bFGF) also improves when encapsulated by liposomes (bFGF-lip) using the pH-gradient method. The study demonstrates that medium dose of bFGF-lip (60 IU ml^{-1}) heals deep second-degree burn model in rats by stimulating fast and high production of tissue collagen, tumor growth factor (TGF)-β1, and proliferating cell nuclear antigen (PCNA) [6]. Various liposomal complexes are applied for drug delivery due to their advantages (Table 1). Two basic problems of liposome (rapid degradation and clearance by the liver macrophages) [7] could be reduced using stealth liposomes coated with materials such as polyoxyethylene, which would prevent opsonization [8, 9], or introducing substances—including cholesterol, various phospholipids with high transition temperature (e.g. distearoylphosphatidylcholine (DSPC)) [10], and polyvinylpyrollidone polyacrylamide lipids [11]—to prolong the circulation time of liposomes in membrane. Incorporation of poly ethylene glycol (PEG) in drugs forms a pseudo-delivery vehicle layer that leads to improved drug solubility, prolonged circulation time, increased drug stability, and decreased proteolytic degradation. It retards mononuclear phagocyte system (MPS) recognition and increases circulation time by forming a hydration layer that inhibits hydrophobic and electrostatic interactions with plasma proteins [12]. Liposomes coated with PEG are applied in the chemo-immunotherapy of cancer in novel co-encapsulation of alendronate and doxorubicin [13]. Another novel pegylated liposome system—called DAFODIL (Doxorubicin And 5-Flurouracil Optimally Delivered in a Liposome)—inarguably offers reduced toxicity and improved therapeutic efficacy in comparison to free drug administrations. In this regard, a decrease by more than 90% of tumor growth in murine 4T1 mammary carcinoma cells is observed following the *in vivo* delivery of remarkably low synergistic doses of the drug pair [14]. Propylene glycol could also enhance solubility of the inner water phase and hydrophilicity of phospholipid bilayers of deformable liposomes. As a consequence, fibrauretine (FN)-loaded propylene glycol-deformable liposome complex, which has a high entrapment capacity, noticeably improves the permeability of FN through the vaginal mucosa [15].

Liposomes can also be conjugated with antibody to fight against a tumor antigen. In this regard, CD^{74+} B-cell malignancies and severe combined immunodeficient (SCID) mouse models engrafted with Raji cells were treated by liposomes. It was shown that milatuzumab-targeted dexamethasone liposomes could increase the killing of transformed CD^{74-} positive B-cells in comparison to CD^{74-} negative T-cells [16]. Additionally, liposomes conjugated with antibody can contain an enzyme in order to convert active cytotoxic drugs only inside the tumor (Antibody-directed enzyme prodrug therapy (ADEPT)) [17].

A multi-functional liposome-mediated drug delivery system based on the interconnection of biomaterial tagged to biotin (Bi) and streptavidin-tagged liposome (SAL) is proposed for many leukemia cells and also mouse xenograft models. In this system, a strong linkage

between SAL, Bi-tagged biomaterials, and surface proteins expressed in cell lineages is formed along with drug cell uptake. Calcein- and cytosine arabinoside (AraC)-encapsulated SALs, combined with Bi- G-CSF, Bi-anti-CD33, or Bi-anti-CD7, reveal effective fluorescent rate of cell-binding or cell lysis [18].

The interaction between liposomal formulations and the inhalation technology for the purpose of delivering drugs in the aerosol form is also interesting. Further, it develops dry powder liposomes like ARIKACE® (Insmed, NJ, USA) as a liposomal amikacin and Pulmaquin™ (Aradigm Corp., CA, USA) as a liposomal ciprofloxacin [19]. Arikace administered once daily for 28 days shows tolerability, safety, and efficacy in patients with cystic fibrosis and virulent pseudomonas aeruginosa infection [20].

In another study on rats, the effect of PEG_{2000}-conjugated hexadecylcarbamoylmethyl hexadecanoate (HDAS-PEG) available in the outer layer of liposomes is investigated over circulation time. The results after 24 hours of administration exhibit less than 1% of ^{99m}Tc-plain liposomes. Around 4.8% of ^{99m}Tc-distearoylphosphatidylethanolamine (DSPE)-PEG-liposomes are preserved in blood whereas 28% of ^{99m}Tc-HDAS-PEG-liposomes remain in blood [21].

In a local drug delivery study, polymeric microspheres or liposomes loaded with doxorubicin (sphere-dox or lipo-dox) were integrated with thermo-reversible gelation polymer (TGP) to provide a sustained release system of doxorubicin. The results show dox release for up to 30 days *in vitro*, and also until Day 32 and Day 38 respectively for sphere-dox and lipo-dox forms in subcutaneous glioma tumor in nude mice [22]. The thermo-sensitive gelation is also applied in another sustained release system known as gelliposomes (GLs). These liposomes include poloxamer solutions to intensify the interior aqueous phase. The gelliposomes are prepared using a thin-film method for encapsulating cytosine arabinoside. The sol–gel transition temperatures of GLs are related with molecular weight and concentration ratio of poloxamers (407 vs. 188). Apart from sustained release, prevention of phagocytosis is also observed for GLs [23].

Combination of the thermosensitive liposomes and short-chain sphingolipids has surprisingly results. Pretreatment of melanoma cells with C_8-glucosylceramide-enriched drug-free liposomes before treatment with doxorubicin-loaded thermosensitive liposomes were found to improve doxorubicin uptake and cytotoxicity. Hence, this novel two-step drug-delivery approach can be potentially beneficial for the intracellular delivery of cell-impermeable chemotherapeutics [24].

Bacteria and liposome could also be combined by an antibody-binding technique to create a functional drug delivery system with high mobility [25].

In another sustained release system, integration of hydrogel chitosan-glycerophosphate and liposome reveals promising results. Hydrogel liposomes (DSPC/cholesterol/DOPE) labeled with ^{99m}Tc-hexamethylpropyleneamineoxime (^{99m}Tc-HMPAO) injected into mouse peritoneum appear in a two-phase blood profile with a descending trend in early hours because of gel formation and then show an ascending trend because of gel disappearance [26].

One of the obstacles of drug delivery is the body's natural barriers (skin and eyes). However, the microfluidic method allows painless and noninvasive delivery of molecules into porcine dermal tissue using nearly monodisperse liposomes within the size range of interest (approximately 40 nm or less) [27]. Skin permeability coefficients of anesthetic butamben (BTB) could be greatly improved by using liposomal gel formulations compared to plain (conventional liposomes) gel (without liposome) formulations (1.02 ± 0.23 for 10% BTB vs. 2.96 ± 0.77 for 10% conventional liposomes and 4.14 ± 0.9 for 10% elastic [gel] liposomes) [28]. The ophthalmic delivery system for tacrolimus (FK506) with approximately 100 nm of nanoliposomes containing bile salts (sodium taurocholate and sodium glycocholate) also reduces toxicity and vastly improves corneal permeability [29]. Recently, flurbiprofen-loaded deformable liposomes (FP-DL) have been administered via a modified ethanol injection method. These liposomes were coated with chitosan (CS) and offered as an ocular drug delivery system for long-term pre-corneal retention, and improved transcorneal penetration and absorption. As a result, coating with 0.1% CS shifted the zeta potential from negative to positive. The given permeability coefficient values of FP-DL-0.1% CS gauged by isolated rabbit corneas are 1.29, 1.95, and 4.59 times more than that of uncoated FP-DL, conventional liposome, and FP solution respectively. In addition, the *in vivo* pre-corneal retention time is prolonged and ocular irritation test *in vivo* results in no eye injury or abnormal clinical indications [30].

The application of liposomes coated by glycans in immunotherapy has also been investigated. For instance, oligomannose-coated liposomes (OMLs) can be taken up through human peripheral blood monocytes (PBMs) and human peritoneal macrophages (PEMs) with CD_{14+} - CD_{206+}. Subsequently, these cellular vehicles agglomerate liposomes at the micrometastatic foci of the omentum after intraperitoneal injection of gastric cancer cells into mice [31]. Additionally, the targetable galactosylated liposomal vaccine effectively facilitates antigen uptake by dendritic cells (DCs) as compared to unmodified liposomes both *in vitro* and *in vivo*. Further, intranasal

immunization of C57BL/6 mice thrice with ovalbumin (OVA)-encapsulated galactosylated liposomes leads to complete protection against EG7 tumor due to the high production of OVA-specific IgG antibodies in serum. Mouse spleen cells receiving galactosylated liposomes also show significantly increased levels of IFN-γ, IL-4, IL-5, and IL-6 [32].

Recently, sterically stabilized liposomes (SSL) have been represented as having 139nm diameter, a zeta potential of −28.1, and polydispersity index (PDI) of 0.05. These liposomes, incorporating IPA-3, inhibit prostate-cancer cell growth *in vitro* with comparable efficacy to free IPA-3. Analysis of liposomal IPA-3 levels shows that 70% of IPA-3 remain within the liposome after seven days. Interestingly, a two-day/week dose of SSL-IPA-3 could inhibit the growth of prostate xenografts *in vivo* as compared to free IPA-3 [33]. The other novel targeting drug delivery methods include complementary coiled coil formation by peptides E_4 [$(EIAALEK)_4$] of liposome and K_4 [$(KIAALKE)_4$] of zebrafish xenografts of HeLa-K. It enables drug selectivity and efficacy *in vivo*. In other words, cancer proliferation is suppressed better in the xenograft by E_4-liposome-doxorubicin as compared to free doxorubicin [34].

Table 1: Examples of novel compositions or methods used in liposomes fabrication and their advantages for drug delivery

Novel compositions or methods used in liposomes	Cells or tissues delivered	Use for	Advantages	Reference
bFGF-lip, pH gradient method	Skin	Wound-healing therapies	Stability of bFGF improved. Medium dose of bFGF-lip heals deep second-degree burns model in rats by stimulating fast and high production of tissue collagen, (TGF)-β1, and PCNA	Xiang *et al.* [6]
Polyoxyethylene, folic acid, Pep-1 membrane penetrating peptide	Human keratinocyte (HaCaT) and cervical cancer (HeLa) cells	Cancer therapy	Steric hinderance prevents opsonization and prolongs the circulation time. In addition, targeted treatment of folic acid receptor–positive tumors with high translocation capability takes place.	Kang *et al.* [9]
Cholesterol, phospholipids with high transition temperature (e.g. DSPC)	Eyes	Cancer therapy	Prolongs the lifetime of Avastin in the eyes	Shih *et al.* [10]
PEG	Murine 4T1 mammary carcinoma tumors	Cancer therapy	Cardiotoxicity reduced and therapeutic efficacy improved in comparison to free drug administration. A decrease by more than 90% of tumor growth is seen in vivo.	Camacho *et al.* [14]
Propylene glycol	Vagina mucosa of rabbits and rats as well as human vaginal epithelial cells (VK2/E6E7)	Gynecological inflammation	Solubility of inner water phase and hydrophilicity of phospholipid bilayers of FDL enhanced. As a result, Entrapment capacity and permeability of FN improved.	Li *et al.* [15]
Milatuzumab	Raji-SCID xenograft model, primary chronic lymphocytic leukemia cells	Cancer therapy	Dexamethasone liposomes could increase selective killing of transformed CD^{74-} positive B-cells in comparison to CD^{74-} negative T-cells.	Mao *et al.* [16]
SAL/ Bi-tagged biomaterials	Human leukemia cells and mouse xenograft models	Cancer therapy	Targeted cell uptake of Calcein and AraC-encapsulated SALs combined with Bi- G-CSF, Bi-anti-CD33 or Bi-anti-CD7 leads to effective fluorescent rate of cell binding or cell lysis.	Chen *et al.* [18]

Aerosolized liposome	Lung	Cystic fibrosis and virulent Pseudomonas aeruginosa infection	Liposomal amikacin shows tolerability, safety, and efficacy.	Clancy *et al.* [20]
HDAS-PEG	Circulatory system of the rat	Study of circulation persistence	Non-phosphoryl HDAS-PEG desorbs outer layer of liposomes at nearly half the rate of DSPE-PEG. It greatly lowers liposome-induced complement activation, Thus, after 24 h of administration, 28% of ^{99m}Tc-HDAS-PEG-liposomes remained in blood.	Nag *et al.* [21]
TGP	Glioma cells and human subcutaneous glioma tumor in nude mice	Cancer therapy	Doxorubicin is released for up to 30 days *in vitro*, and until Day 38, for lipo-dox form in vivo, along with inhibition of growth.	Arai *et al.* [22]
Poloxamer 407, poloxamer 188	Sustained release, in vitro	Cancer therapy	Physical stability of gelliposome-encapsulating cytosine arabinoside leads to sustained release and prevented phagocytosis.	Zhang *et al.* [23]
Short-chain sphingolipids enriched drug-free liposomes/ Thermosensitive liposomes	Melanoma cells	Cancer therapy	Cell internalization of doxorubicin and its cytotoxicity improves by the two-step drug delivery approach.	Haeri *et al.* [24]
CS-glycerophosphate	Injected to mouse peritoneal cavity	Study of controlled release	Hydrogel liposomes appear a two-phase blood profile with a descending trend in early hours because of gel formation, and then an ascending trend because of gel disappearance.	Alinaghi *et al.* [26]
Microfluidic method	Porcine dermal tissue	Passive transdermal drug delivery	Painless and noninvasive delivery of molecules along with size-dependent passive uptake occurs.	Hood *et al.* [27]
Liposomal gel formulations	Skin	Passive transdermal drug delivery	Skin permeability coefficients of anesthetic BTB could be highly improved by using the liposomal gel formulations.	Cereda *et al.* [28]
Bile salts (sodium taurocholate and sodium glycocholate)	Human corneal epithelial cells and the rabbit cornea	Inhibition of immunological rejection	Ophthalmic delivery system for tacrolimus reduces toxicity and improves corneal permeability vastly after graft.	Dai *et al.* [29]
CS	Rabbit cornea	Ocular inflammation	Coating with 0.1% chitosan shifts the zeta potential from negative to positive. The permeability coefficient of FP-DL-CS is improved. In addition, the in vivo pre-corneal retention time is prolonged and ocular irritation test in vivo leads to no eye injury or abnormal clinical indications.	Chen *et al.* [30]
Oligomannose	Human gastric cancer cell and tumor	Immunotherapy of cancer	OMLs could be taken up through human PBMs and PEMs with CD_{14+} - CD_{206+}. These cellular vehicles agglomerate liposomes at the micrometastatic foci of the omentum of mice and tumor foci in the surgically resected human omentum ex vivo.	Matsui *et al.* [31]

OVA- Galactose	Dendritic cells	Immunotherapy of cancer	Antigen uptake is effectively facilitated by dendritic cells. Intranasal immunization of C57BL/6 mice leads to complete protection against EG7 tumor. Mouse spleen cells receiving galactosylated liposomes also showed significantly increased levels of IFN-γ, IL-4, IL-5 and IL-6.	Jiang *et al.* [32]
SSL	PC-3 human prostate cancer cell and xenograft into mouse	Cancer therapy	SSL incorporating IPA-3 inhibits prostate cancer cell growth *in vitro* with comparable efficacy to free IPA-3. 70% of IPA-3 remain within the liposome after seven days. A two-day/week dose of SSL-IPA-3 could inhibit the growth of prostate xenografts *in vivo*, as compared to free IPA-3.	Al-Azayzih *et al.* [33]
Coiled Coil Peptide E_4 [$(EIAALEK)_4$],	Zebrafish xenografts of HeLa-K	Cancer therapy	Complementary coiled coil formation by peptides E_4 [$(EIAALEK)_4$] of liposome and K_4 [$(KIAALKE)_4$] of zebrafish xenografts of HeLa-K enables drug selectivity and efficacy.	Yang *et al.* [34]

Abbreviations: bFGF-lip, basic fibroblast growth factor; TGF)-β1, tumor growth factor-β1; PCNA, proliferating cell nuclear antigen;DSPC, distearoylphosphatidylcholine; PEG, poly ethylene glycol; DAFODIL, doxorubicin and 5-flurouracil optimally delivered in a liposome; FDL, fibrauretine loaded propylene glycol-deformable liposome; FN, fibrauretine; SCID, severe combined immunodeficient; SAL, streptavidin-tagged liposome; Bi, biotin; AraC, arabinoside; HDAS, hexadecylcarbamoylmethyl hexadecanoate; DSPE, distearoylphosphatidylethanolamin; TGP, thermo reversible gelation polymer; lipo-dox, liposome-doxorubicin; CS, chitosan; BTB, butamben; FP-DL, flurbiprofen-loaded deformable liposomes; PBMs, peripheral blood monocytes; PEMs, peritoneal macrophages; OMLs, oligomannose-coated liposomes; OVA, ovalbumin; SSL, sterically stabilized liposomes; E_4, $[(EIAALEK)_4]$; K_4, $[(KIAALKE)_4]$;

Nanotechnology and gene delivery

1. Nanoparticles

In general, viral vectors used for gene transfer have limitations of safety issues, stimulation of immune system, and antibody production against these vectors [2]. Comparatively, functionalized fullerenes are scrutinized as transfection vectors to deliver exogenous DNA into cells and examined for their ability to mediate gene transfer [35, 36]. Even though the first-generation fullerenes are promising, they also show high cytotoxicity [37]. The study indicates that only two positively charged fullerene C_{60} derivatives (octa-amino and dodeca-amino derivatives) transfect exogenous DNA efficiently *in vitro* [38]. However, a water-soluble cationic tetraamino fullerene also shows promising results *in vivo*. The tetra (piperazino) fullerene epoxide (TPFE)–siRNA complexes could release the mouse toll-like receptor 4 (TLR4)-targeted siRNA into lung cells and knock down the TLR4 mRNA, and are subsequently removed rapidly from the lung after siRNA delivery. Hence, neutrophil accumulation induced by lipopoly saccharide (LPS) injection is suppressed in the lung [39].

Regardless of the positive effect of high-field/high-gradient magnets and dynamic magnetic fields on overall transfection levels of magnetic nanoparticles (MNPs) [40], various hybrid formulations also help this purpose. For instance, PEI-magnetic Fe_3O_4 nanoparticles transfect successfully enhanced green fluorescent gene (EGFP) in PK-15 cells using external magnetic field [41]. In another study, a gene delivery system called magnetofection, which uses MNPs coated with PEG and branched PEI (bPEI), was found to increase transfection immensely in human umbilical vein endothelial cells (HUVEC), even in serum condition [42]. An *in vivo* study following intrathecal injection of magnetic nanoparticle/PEI complexes shows targetable delivery as well [43]. However, as compared to naked DNA, MNPs ($TransMAG^{PEI}$) complexed to naked DNA or liposome including lipofectamine 2000 or cationic lipid 67/plasmid DNA show poor gene delivery into the airway in vivo [44, 45]. Ingenious combination of magnetic Fe_3O_4 nanoparticles coated with poly (styrene) sulfonate with PAMAM (poly amido amin) dendrimers-pDNA complexes (dendriplexes), through electrostatic interactions, also potentially develops a gene delivery vehicle comparable to dendriplexes alone. Yield of gene expression in NIH 3T3 cells is highly dependent on the dendrimer generation, the amine to phosphate group (N/P) ratio, and the pDNA concentration. In this regard, the best system is constituted by dendrimers of Generation 6 at N/P ratio of 10 [46].

CpG oligodeoxynucleotide (CpG-ODN) can also be delivered through MNPs to induce apoptosis. A novel targeted-delivery system—including a Fe_3O_4 magnetic core, a 3-Aminopropyl triethoxysilane (APTS) interlayer, and a cationic PAMAM dendrimer—could be attached to CpG-ODN molecules due to high positive charges on the nanoparticles surface. These nanoparticles, with average size of 40 ± 10 nm, increase the accumulation of CpG-ODN molecules in MDA-MB231 and SKBR3 breast tumor cells and activate Toll-like receptor 9 (TLR9), generating a signal cascade for cell death [47].

Another interesting approach based on nanomaterial-mediated gene delivery is the use of nanotubes. In this regard, ammonium group-functionalized multiwalled carbon nanotubes (MWCNTs) are considered as a carrier for developing DNA vaccine to control virus infection. Potential properties of nanotubes—such as making condensed DNA, covering the cell surface, penetrating the cell membrane, and expressing transgene—are confirmed using agarose gel shift assay, SEM and TEM microscopic images, and real-time PCR respectively. Consequently, functional groups and charge ratio are two determinant factors regarding the transfection efficiency and expression of plasmid DNA (pEGFP-*vp5*) in Ctenopharyngodon idellus kidney (CIK) cells [48]. In another study, the combination of PEI-grafted MWCNTs and chitosan substrate efficiently transfected model gene of enhanced green fluorescence protein (EGFP) into hard-transfected cells such as bone marrow mesenchymal stem cells (BMSCs), along with low toxicity under optimum condition [49]. Similarly, Quantum dots (QDs) are also applied in gene delivery into human mesenchymal stem cells (hMSCs). It is observed that PEI coating alters negative charges of QDs and leads to various size-bundled QD nanoparticles. Further, the largest QD bundled NPs—called QD655—have significantly higher gene transfection efficacy and greater expression than single-type QDs and the smaller-bundled QD nanoparticles [50].

2. Liposomes

Like other nanoparticles, liposomes also can be applied effectively in gene delivery (Table 2). Cationic liposomes (CLs) are synthetic nanocarriers of nucleic acids in gene delivery and gene silencing [51]. Recently, a liposomal complex was fabricated to improve the delivery efficiency of microRNA-145 for cancer therapy. Consequently, transfection efficiency increased with a DNA-condensing agent like protamine. Further optimized conditions included a molar ratio of DOTAP/cholesterol of 3:1 for the preparation of the liposomes, 5% glucose as the hydration medium, and a weight ratio of DOTAP/protamine/DNA of 3:0.5:1. In conclusion, the overexpression of miR-145 inhibits growth of the HepG2 cells and downregulates expression of CDK6, c-myc, cyclinD1, and Sp1 transcription factor [52]. Hyaluronic acid (HA) biopolymer—as an anionic mucoadhesive—could coat cationic liposomes constituted of egg phosphatidylcholine, DOPE and DOTAP with 2:1:1 molar ratio respectively. Consequently, the pDNA/CL/HA nanocomplexes with the different HA amounts efficiently deliver reporter gene (luciferase) into HeLa cells [53]. In another study, liposomes (>100nm) incorporated with polyethylene glycol and galactose targeted liver Kupffer cells, where N-acetylgalactosamine injected one min prior to the administration of Gal-PEG-liposomes reduced rapid absorption of these cells. Incorporation of monomethoxypoly (ethyleneglycol)-distearoylphosphatidylethanolamine (PEG-DSPE) in the Gal-PEG_{10}- liposomes also increased the effect of the galactolipid regarding spleen uptake [54]. Thus, gene therapy may be attempted with such liposomal nanoparticles for various liver disorders such as Wilson's illness, hereditary hemochromatosis, and α1-antitrypsin deficiency.

Table 1: Some novel liposomal complexes and other nanoparticles used for gene delivery and their advantages

Complexes	Cells or tissues delivered	Use for	Advantages	Reference
CL/protamine/miR-145/5% glucose	HepG2 cells	Cancer therapy	Transfection efficiency increases, the overexpression of miR-145 inhibits growth and downregulates expression of CDK6, c-myc, cyclinD1, and Sp1 transcription factor	Tao *et al.* [52]
pDNA/CL/HA	HeLa cells	Cancer therapy	Nanocomplexes with different HA amounts as anionic mucoadhesive efficiently delivered Luciferase gene.	Balbino *et al.* [53]
Liposome/PEG/ galactose/DNA	Injected intravenously to rat	Liver disorders	Nano liposome targets liver cells effectively owing to their rapid absorption by liver Kupffer cells via galactose receptors.	Shimada *et al.* [54]
TPFE–siTLR$_4$ complex	intravenous injection into mice	Lung diseases	Nanocomplexes could release the mouse toll-like receptor 4 (TLR$_4$)-targeted siRNA, knock down the TLR$_4$ mRNA, and be subsequently removed rapidly from the lung after siRNA delivery. Hence, neutrophil accumulation induced by LPS intratracheally injection and the LPS-TLR$_4$ signaling pathway is suppressed in the lung.	Minami *et al.* [39]

PEI-magnetic Fe_3O_4 nanoparticle- pDNA EGFP	PK-15 cells	Study of controlled gene delivery	A large number of DNA are condensed around the surface of MNPs. EGFP gene is transfected successfully using external magnetic field into cytoplasm and nucleus.	Wang *et al.* [41]
MNP/PEG/ bPEI/DNA	HUVEC	Study of the function and pathology of endothelial cells	Magnetofection increases targeted transfection immensely, even in serum condition. Expression of PAI-1 responsible for different vascular disorders such as vascular inflammation and atherosclerosis is inhibited. Nanostructure reveals great degree of anti-biofouling, cell viability, and serum stability.	Namgung *et al.* [42]
MNPs complexed to lipofectamine 2000 or cationic lipid 67/ pDNA liposome, MNPs-naked DNA	Airway epithelial cultures, murine nasal epithelium, in vivo	Study of airway gene transfer for diseases such as cystic fibrosis.	Despite improved transfection efficiency in vitro, it shows poor gene delivery in vivo.	Xenariou *et al.* [44]
Magnetic Fe_3O_4 nanoparticles coated by poly (styrene) sulfonate - PAMAM dendriplexs	NIH 3T3 cells	DNA transfection studies	Nanocomplex is demonstrated as a potential gene transfer vehicle. Yield of gene expression is highly dependent on the dendrimer generation, N/P ratio, and the pDNA concentration. The best system is constituted by generation of 6 dendrimers at N/P ratio of 10.	Xiao *et al.* [46]
Fe_3O_4 magnetic core/ 3-APTS/cationic PAMAM dendrimer/ CpG-ODN	MDA-MB231 and SKBR3 breast tumor cells	Cancer therapy	These nanoparticles with the average size of 40 ± 10 nm increase the accumulation of CpG-ODN molecules in cells and activate TLR_9, generating a signal cascade for cell death.	Pourianazar *et al.* [47]
MWCNTs-NH_3^+ / pEGFP-vp5	CIK cells	Control of virus infection (CGRV)	Potential properties of nanotubes such as making condensed DNA, covering the cell surface, penetrating the cell membrane, and expressing transgene are confirmed. Functional groups and charge ratio are two determinant factors of the transfection efficiency and expression of plasmid DNA (pEGFP-vp5) as DNA vaccine.	Liu *et al.* [48]
PEI/MWCNTs/CS/pEGFP	BMSCs	Regenerative medicine	Nanostructure efficiently transfects model gene into cells that are hard to transfect, along with low toxicity under optimum condition. It leads to more sustained expression of the EGFP in short-term cultures.	Moradian *et al.* [49]
PEI-QDs/DNA	hMSCs	Cell therapy	PEI coating alters negative charge of QDs leading to various size-bundled QD nanoparticles. The largest QD bundled nanoparticles—called QD655—have significantly higher gene transfection efficacy and greater expression than single-type QDs and the smaller-bundled QD nanoparticles.	Yang *et al.* [50]

Abbrevations: CLs, cationic liposomes; miR-145, microRNA-145; pDNA, plasmid DNA; HA, hyaluronic acid; PEG, poly ethylene glycol; TPFE–$siTLR_4$, tetra (piperazino) fullerene epoxide- toll-like receptor 4 (TLR4)-targeted siRNA; LPS, lipopoly saccharide; PEI, poly ethyleneimine; EGFP, enhanced green fluorescent protein; MNPs, magnetic nanoparticles; bPEI, branched poly ethyleneimine; HUVEC, human umbilical vein endothelial cells; PAMAM, poly amido amin; APTS, aminopropyl triethoxysilane; CpG-ODN, CpG oligodeoxynucleotide; MWCNTs-NH_3^+, ammonium group-functionalized multiwalled carbon nanotubes; CIK, ctenopharyngodon idellus kidney; CGRV, carp grass reovirus; BMSCs, bone marrow mesenchymal stem cells; QDs, quantum dots; hMSCs, human mesenchymal stem cells;

CONCLUSION

Nano delivery systems of liposomes have the ability to overcome problems of drug resistance in target cells and to facilitate the movement of drugs and genes across membrane barriers. It is concluded that the small size, improved solubility, customized surface, and multi-functionality of nanoparticles would lead to better bio-compatibility, bio-accessibility, and efficacy under different conditions. However, better understanding of the fate of the drugs delivered by nanoparticles to the nucleus and other sensitive cell organelles is necessary for dealing with virulent diseases.

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