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Emerging Trends and Recent Development in the Synthesis of Non-Viral Cationic Systems for Targeted Gene Delivery Applications

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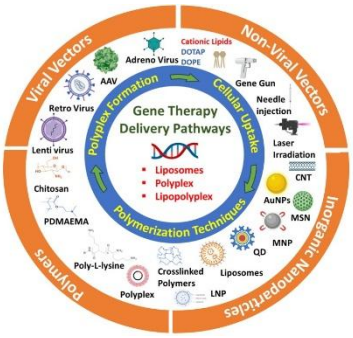
Abstract: The development of a safe and efficient nucleic acid delivery system remains a major challenge in gene therapy. Viral and non-viral vectors are widely explored: Viral vectors often achieve high transduction efficiency, but are limited by high immunogenicity, oncogenic risk, and complex production processes, whereas non-viral vectors are comparatively safer, easily scalable, and structurally versatile; however, their clinical application is limited by low transfection efficiency. To overcome these challenges, hybrid strategies that combine different non-viral systems have emerged as a prominent approach for safer therapeutic gene delivery. Among these systems, lipid-based and polymer-based cationic systems have been extensively explored due to their tunable chemical structures and high nucleic acid loading capacity, enabling the design of multifunctional cationic systems for efficient and safe delivery. Hence, this review focuses on the synthesis and design of multifunctional cationic systems, highlighting their potential to overcome current barriers in non-viral gene delivery and to advance next-generation therapeutic platforms.



Keywords: *Gene delivery, viral vectors, non-viral vectors, lipids, polymers, lipid-polymer conjugates*

Abbreviations: DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid; AONs: Antisense oligonucleotides; DOTMA: 2,3-bis[oleyl]oxipropyl trimethylammonium chloride; DOTAP: [1,2-bis-oleoyloxy)-3-trimethylammonio)propane; AC-Chol: Dimethylhydroxyethylaminopropane carbamoyl cholesterol; MC-Chol: Monomethylaminoethane carbamoyl cholesterol; DOPE: Dioleylphosphatidylethanolamine; DOGS: Dioctadecylamidoglycylspermine; BGTC: Bis-guanidinium-tren-cholesterol; DC-Chol: 3β-[N-(N',N'-dimethylaminoethyl)-carbamoyl] cholesterol; DODAG: *N', N'*-dioctadecyl-N-4,8-diaza-10-aminodecanoyl glycine amide; DEAE: Diethylaminoethyl; PEI: Polyethyleneimine; PLL: Poly(L-lysine); DMAEMA: Dimethylamino ethylmethacrylate; PAEs: Poly(β-amino esters); PAA: Polyallylamine; PEG: Polyethylene glycol; CRP: Controlled radical polymerization; RDRP: Reversible deactivation radical polymerization; RAFT: Reversible addition fragmentation chain transfer polymerization; ATRP: Atom transfer radical polymerization; NMP: Nitrogen mediated polymerization; PPGMA: Poly(propylene glycol methacrylate); MDO: 2-Methyl-1,3-dioxepane; PA: Propargyl acrylate; BMDO: 5,6-benzo-2-methylene-1,3-dioxepane; PEO: Poly(ethylene oxide); EtBr: Ethyl bromide; DDMAT: 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid; AIBN: Azobisisobutyronitrile; LAMA: Lipoic acid methacrylate; nBMA: n-Butyl acrylate; EMA: Ethyl methacrylate; MMA: Methylmethacrylate; CPAETC: 4-Cyano-4[[(ethylthio)thioxomethyl]thio] pentanoic acid; ACVA: 4,4′-Azobis(4-cyanovaleric acid; NVF: *N*-vinyl formamide; NVP: *N*-vinyl pyrrolidone; PI: photoiniferter reversible addition fragmentation chain transfer polymerization; Xan: O-ethyl xanthate; PVAm: Polyvinyl amine; SPPS: Solid phase peptide synthesis; GA: Gallic acid; PHBA: p-Hydroxybenzoic acid; TPP: Triphenyl phosphonium; AuNs: Gold nanostars; CS: Chitosan; CD: Cyclodextrin; bPEI: Polyethyleneimine; PABA: p-Aminobenzoic acid; DCD: Dicyandiamide; Boc-DAB: *N*-Boc-1,4-diaminobutane; OA: Oleic acid; CCDs: Cationic carbon dots; PEHA: pentaethylenehexamine; PHP: Poly(2-hydroxypropenimine); UMMD: Ultrasound-mediated microbubble destruction; GMP: Good manufacturing practice.

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1. Introduction

1.1. Gene Therapy

Gene therapy is an innovative medical technique that modifies defective or missing genes to treat or prevent diseases.[1] These genes are composed of segments of deoxyribonucleic acid (DNA), which is made up of nucleotides. DNA contains the instructions for cells to produce proteins, which perform various functions in the human body. The act of transferring these exogeneous genetic materials, such as DNA or ribonucleic acid (RNA), into target cells to enable gene expression is known as gene delivery. Gene delivery must enter the host cell's genome to cause gene expression. In gene therapy, DNA and RNA gene molecules are competent of altering the defective genes, modifying missing genes, and silencing muted genes.[2] Gene therapy is widely explored to treat a range of diseases, including both inherited and acquired conditions.[3] Numerous inherited ailments, including genetic disorders, cardiovascular diseases, cancerous tumours, diabetes mellitus, infectious diseases, and neurological problems, can be treated with gene therapy.[4] These naked therapeutic genes are quickly degraded by nucleases and have low cellular uptake. Hence, developing safe and efficient gene carriers remains crucial for advancing gene therapy.[5]

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Figure 1 illustrates gene delivery into a target cell where naked DNA, viral vectors or growth factors, enter the cell and reach

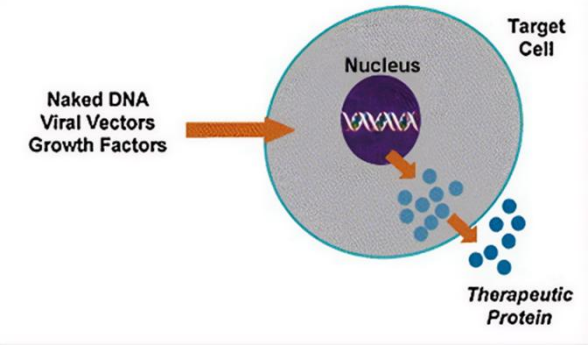


Figure 1. *Gene delivery. Adapted with permission from ref 3. Copyright (2024) Elsevier B.V.*

the nucleus and lead to the expression, leading to a release of therapeutic proteins.[3]

With the development of fundamental concepts of gene therapy and their need for efficient gene delivery systems, it is necessary to understand how gene therapy has evolved over the years. The ongoing development of gene therapy throughout the years has been remarkable from early discoveries to the recent advancements in therapeutic products. Thus the **Figure 2** highlights the major advancements over the years in gene therapy.[6]

Despite several gene delivery technologies which are developed over the past 3 decades, no universally applicable technology has been established for gene therapy.[7] Gene therapy can be implemented through several therapeutic strategies, but the strategies depend on the type of genetic modification to treat diseases. The therapeutic strategies are broadly classified into DNA-based and RNA-based approaches.[1] DNA-based methods include gene editing techniques such as CRISPR-Cas9, TALENs, gene augmentation, which introduces a functional gene.[8,9] RNA-based therapies include antisense oligonucleotides (AONs) and small interfering RNA (siRNA), which regulate gene expression at the post-transcriptional level.[10] Both these approaches offer versatile strategies for the treatment of genetic disorders. The classification of the gene delivery strategies is listed in **Figure 3**.[1]

Although gene delivery strategies outline the approach for gene modification, their effectiveness relies on suitable gene delivery vehicles that act as an efficient transport system to deliver the genetic materials into the targeted cells which are known to be as the delivery vehicles.

1.2. Classification of Gene Delivery Vectors

These vehicles are broadly classified into viral (retroviral, adenoviral, adeno-associated) and non-viral vectors (liposomes, polyplexes), which are widely used for gene transduction and transfection respectively. Each has their own advantages and disadvantages,[11] but they are not currently ideal for achieving safe and efficient delivery along with stable and sufficient gene expression. The brief classification of these vectors is shown in **Figure 4**.[1]

1.2.1. Viral Vector

Ongoing advances such as gene delivery, oncolytic virotherapy and Crisper Cas9 offer a successful therapeutic pathway.[12] The most widely utilized gene delivery systems are those that are on viruses, which were the first vectors to be investigated and employed. These are the most efficient method for delivering genes to specific cell types or tissues, enabling therapeutic gene expression.[7] Viruses are used for their potential to transport genes to cells, allowing for temporary or permanent expression. These viruses include adreno, adeno-associated, retro, g-retro, lenti, pox, baculo, and herpes simplex viruses.[13] When selecting a virus for clinical usage, the considerations widely include effectiveness of transgenic expression efficiency, production simplicity, safety profile, stability and toxicity.[14] These vectors are most generally used for gene transfection due to their high transfection efficiency in vivo and also in immunization.[15] Even though there are some advantages in using a virus as a gene delivery vehicle, there are certain limitations that are widely represented in **Table 1**.[16]

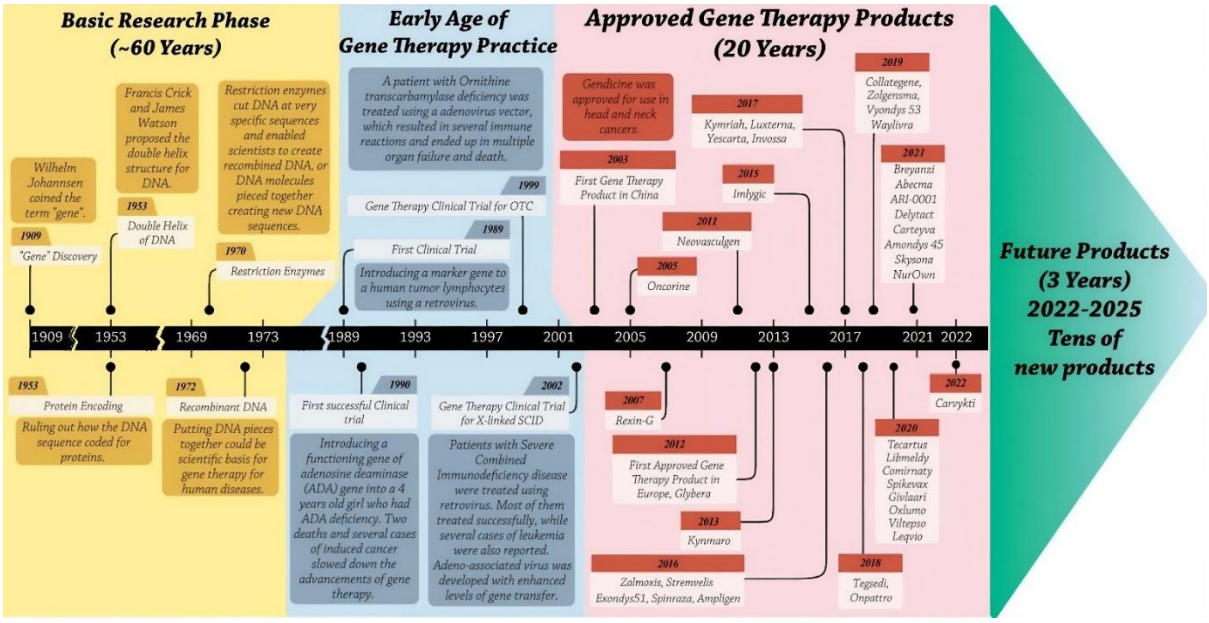


Figure 2. *Historical timeline for gene delivery applications. Adapted with permission from ref 6. Copyright (2022) Elsevier Masson SAS.*

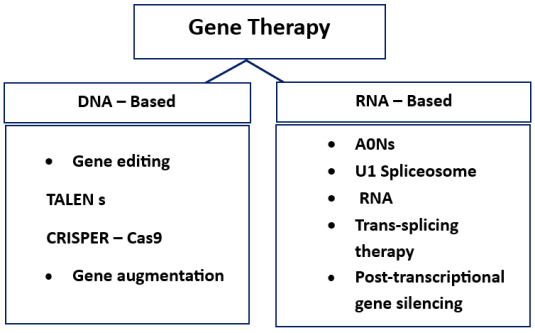


Figure 3. *Classification of gene therapy approaches based on nucleic acid type.*

1.2.2. Non-viral Vector

Recent clinical experiments have emphasized the risks associated with utilizing these viruses to transport and integrate DNA into host cells. To overcome the risks associated with viral delivery techniques, which are mentioned in **Table 1**, alternative delivery systems are employed without the use of viruses, and these are non-viral vectors.[17] Non-viral vectors are generally considered safer than viral ones because they are easier to produce, less expensive, and have a lower potential for insertional mutagenesis and pathogenicity. However, the ineffectiveness of non-viral vectors, cell transport and the temporary expression of their transgenes have prevented the deployment of these vectors to people.[18] These non-viral vectors generally transfers the following types of nucleic acids, such as small DNA (oligonucleotides) or related molecules synthesized chemically, large DNA molecules (Plasmid DNA: pDNA), RNA (Ribozymes, siRNA, mRNA).[19] Thus, the delivery systems are classified into physical and chemical methods. These physical methods disrupt the cell membranes to make them more permeable so that the DNA (or RNA) can enter without the need for an additional carrier. The major principle includes that the mechanical, electrical or pressure-based stress creates a temporary pore in the cell membrane in which DNA enters. These methods are discussed briefly.[20]

2. Physical Methods for Non-viral Gene Therapy

The physical method works by directly disrupting the cell membranes to make them more permeable, so the DNA or RNA can enter without the need for an additional carrier. The major principle includes that mechanical, electrical or pressure-based stress creates a temporary pore in the cell membrane in which DNA enters the cytoplasm and moves towards the nucleus. However, the major limitation includes the damage to the cells, which is difficult for in vivo applications. Some of the methods include electroporation, microinjection, gene gun/biolistic, ultrasound (sonoporation), laser irradiation, and hydrodynamic injection.[21] The principles, advantages, limitations and applications of the physical methods are comparatively discussed in **Table 2**.

3. Chemical Methods for Non-viral Gene Therapy

Chemical methods primarily depend on the synthetic or natural carriers that can bind and protect the DNA, then cross the cellular barriers and reach the nucleus. The carriers are engineered via chemical interactions such as ionic bonding, hydrophobic interactions, and covalent modifications. These carriers protect the DNA from enzymatic degradation and improve the cellular uptake by mimicking natural biomolecules.[22] This also facilitates the endosomal escape after entering the cell. Some non-viral carriers are biodegradable to reduce the toxicity. Some examples of delivery carrier includes the Inorganic nanoparticles, cationic Liposomes (Lipids), cationic polyplexes (Polymers), Inorganic materials and lipid-polymer (Hybrid systems).[23] The ability of nanoparticles to absorb cells allows for the transfer of nucleic acids into living cells. Several different types of inorganic nanoparticles are employed as carriers. Some of the nanoparticles, such as calcium phosphate, carbon nanotubes, silica coated with gold nanoparticles, magnetic quantum dots, iron oxide nanoparticles, strontium phosphate, gold clusters, magnesium phosphate, manganese phosphate and double hydroxides (anionic clays), are employed for DNA transport.[24] One of the main strategies for modifying inorganic nanoparticles for gene delivery is to give them a positive surface charge, allowing them to electrostatically bind with the

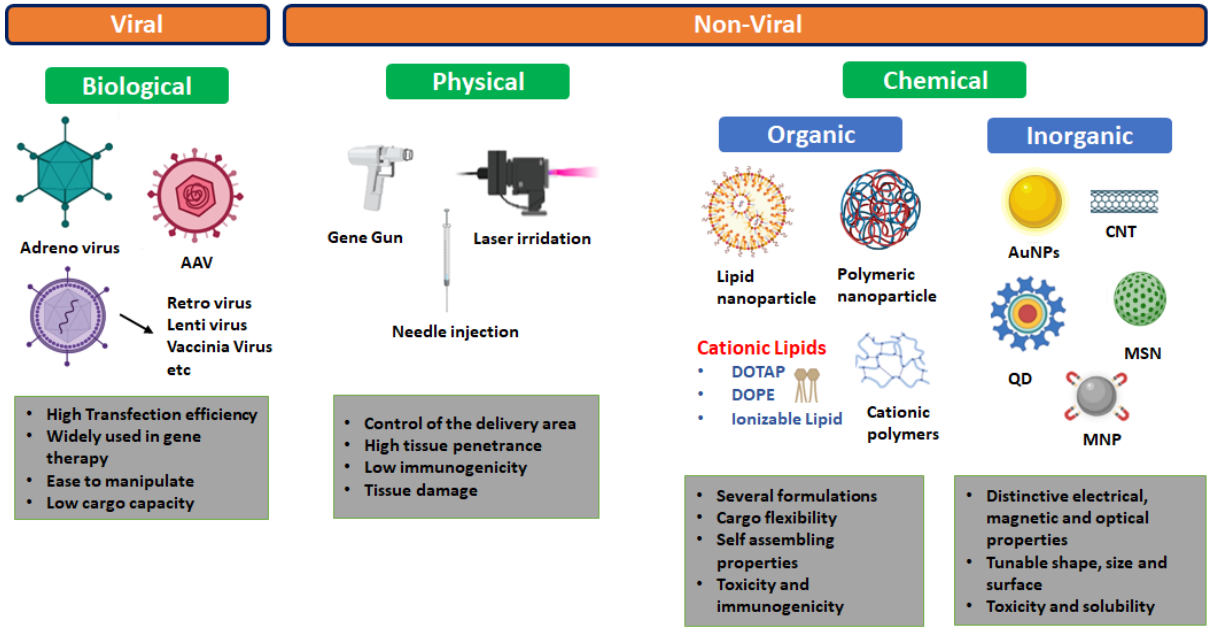
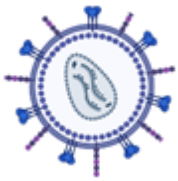
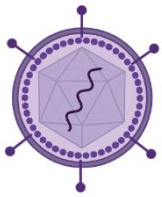
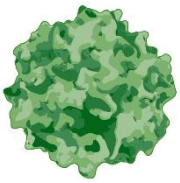


Figure 4. *Classification of different types of vectors for gene delivery. ©Created in BioRender.com*

Table 1. Advantages and disadvantages of different viruses used as viral vectors.

Vectors	Advantages	Disadvantages
Adenovirus 	High transfection efficiency, Transfects proliferating and non-proliferating cells and Substantial clinical experience.	Strong immune responses, Insert size limit of 7.5kb, Difficult to manufacture and maintain internal control, Poor storage characteristics, short duration of expression
Retrovirus 	Fairly prolonged expression, High transfection efficiency, Substantial clinical experience and Low immunogenicity.	Low transfection efficiency in vivo, Insert size limit of 8 kb ex vivo, Transfects only proliferating cells, Difficult manufacture and internal control, Safety concerns (mutagenesis).
Lentivirus 	Transfects proliferating and non-proliferating cells. Transfects haematopoietic stem cells	Very difficult manufacturing and internal control, Poor storage characteristics, Insert size limit of 8 kb, No clinical experience, Safety concerns (origins in HIV)
Adeno-associated virus 	Efficient transfection of a large choice of cell types in vivo, Prolonged expression, Low immunogenicity, Non-pathogenic virus	Difficult in manufacturing and internal control, Limited clinical experience, Safety concerns (mutagenesis), Small packaging capacity
Herpes simplex virus 	Large insert size: 40–50 kb Neuronal tropism, Latency expression, Small genome, no viral genes	Cytotoxic, no targeting, Requires packaging cell line-limited insert size: 5kb, High titers (1010 pfu/mL), but production is difficult.

negatively charged genetic material. In the second approach, the genetic material is covalently attached to the inorganic nanoparticle through a linker molecule. In the third strategy, a cationic amphiphilic polymer associated with the nanoparticle facilitates complex formation with the genetic material.[25] Though their transfection efficacy is quite poor, these inorganic nanoparticles has few advantages over organic ones, such as being easily manufactured being resistant to microbial attack, often having minimal toxicity, and having good storage stability.[26] As this review focuses on multifunctional cationic systems, a critical comparison of non-viral cationic systems is provided in **Table 3** and further discussed in the subsequent sections.

3.1. Cationic Liposomes/Lipid-Based Gene Delivery

Felgner and colleagues introduced the cationic lipid-mediated gene transfer in 1987.[49] In the early 1970s, a lipid-based system, such as liposomes, was initially explored widely in drug delivery applications, including enzyme replacement therapy, cancer chemotherapy and insulin delivery. However, the early study demonstrated that the liposomes could not deliver the DNA into the cells due to their poor DNA encapsulation efficiency, instability and weak cellular interactions. These limitations increased the development of lipid systems, leading to the introduction of cationic lipids for efficient nucleic acid delivery.

These systems are amphiphilic in nature, which are composed of a positively charged hydrophilic head group (for example, quaternary ammonium), hydrophobic domain (e.g., aliphatic chains) and a linear bond (ester bond) and backbone domain enabling the formation of cationic liposomes in combination with neutral co-lipids.[50] The basic domains of a cationic lipid with their role in complexing with nucleic acids are represented in **Figure 5**.[50]

The cationic head groups undergo electrostatic interaction with the nucleic acids to form lipoplexes, which facilitates cellular uptake by partially neutralizing the cell membrane. To correlate with the transfection activity, the hydrophilic headgroups can be classified into six groups based on their structures: quaternary ammoniums, amino acids or peptides, amines, heterocyclic headgroups, guanidiniums etc. There are different cationic lipids with different hydrophilic headgroup structures are represented in **Figure 6**[52] according to their head group domain: A) quaternary ammonium salt, B) primary amine C) secondary amine, D) tertiary amine, E) polyamine, F) guanidinium group G) melamine group.

Table 2. Physical approaches in non-viral delivery systems: Methods, principles, advantages, limitations, and biomedical applications.

Method	Principle	Advantages	Limitations	Application	References
Gene Gun	DNA-coated gold/tungsten particles are accelerated using pressurized helium to penetrate cells	Direct delivery, strong immune response, no carrier required	Tissue damage, limited penetration control	DNA vaccination, intradermal/intratumoral delivery.	36-39
Needle injection	Direct injection of naked DNA into tissue, uptake due to local tissue disruption	Simple, safe and cost-efficient	Low transfection efficiency	DNA vaccines, cardiac and tumour therapy.	39,40
Jet injection	High-speed DNA delivered through pressurized gas (needle-free)	Higher gene expression (~50x) (needle free)	Physical Pain, limited depth control	DNA vaccination, multiple dose delivery.	39
Electroporation	Enter cells through transient pores formed in the cell membrane by electric pulses.	Higher efficiency compared to viral systems.	Tissue damage, difficult in deep organs.	Both in vitro and in vivo.	41,42
Sonoporation	Ultrasound induces cavitation, forming temporary pores in membranes.	Non-invasive enhanced by microbubbles.	Low efficiency without enhancers	Targeted delivery using microbubbles.	43,44
Laser irridation or optoporation	Laser energy is used to create microscopic pores in the cell membrane for DNA delivery.	Selective targeting of a single cell, safe and non-invasive.	High cost, limited efficiency due to limited penetrating capacity.	Limited to exposed regions such as muscle, skin, and intratumor gene transfer.	45,46
Hydrodynamic injection	Rapid injection of a larger volume of DNA solution	Effective in liver delivery	High pressure	Gene delivery in organisms.	47,48

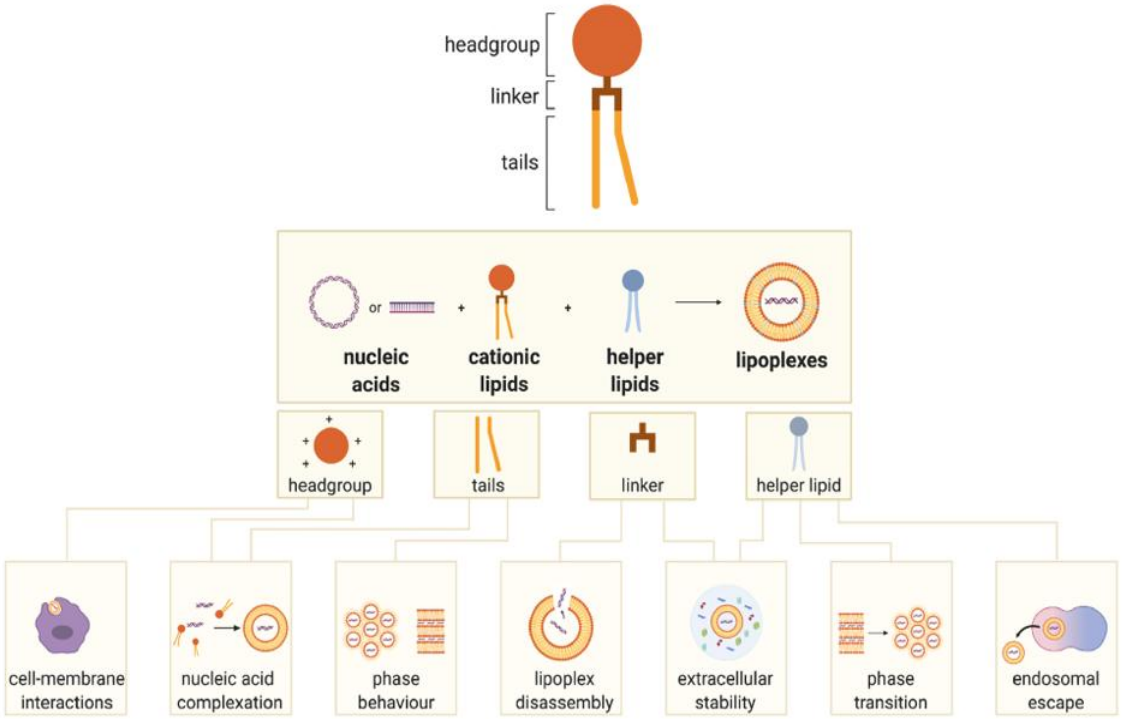
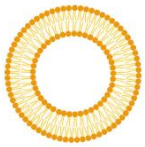
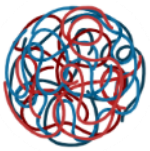
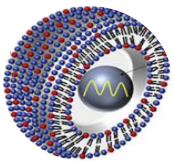
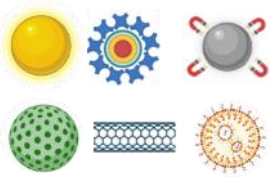


Figure 5. *Schematic representation of the three basic domains of a cationic lipid and their complexation process. Adapted with permission from ref 50. Copyright (2020) Elsevier B.V.*

The primary mechanism of cellular uptake occurs *via* endocytosis, followed by endosomal escape through membrane destabilization, enabling the release of genetic material into the cytoplasm for efficient gene expression. Beyond the mechanism, the structure of cationic lipids plays a critical role in transfection efficiency, where variations in head group charge density, linker, and hydrophobic tail composition significantly influence stability, cellular uptake and endosomal escape.[51] For instance, Felgner and colleagues synthesized the (2,3-bis[oleyl]oxipropyl trimethylammonium chloride

Table 3. Critical analysis of different non-viral gene delivery systems.

Non-Viral System	Characteristics	Limitations	Application	Efficiency and Toxicity metrics	References
Liposomes 	Amphiphilic vesicle structure encapsulates both hydrophilic and hydrophobic substances, non-immunogenic.	Poor stability, flocculation, coalescence, drug leakage and unstable systems.	Pharmaceutical, Cosmetic, food, drug/gene delivery.	It has High encapsulation efficiency with high solubility and low toxicity.	27,28
Polyplexes 	Protect nucleic acid from enzymatic degradation, targeted release, non-immunogenic, High water solubility, easy to modify and tailored in size and composition.	Non-biodegradable, and not all polymers have endosome-lytic characteristics	Receptor Targeted Delivery, agricultural, drug/gene delivery	Enhanced transfection and toxicity concerns, especially with PEI	29,30
Lipopolyplexes 	Core-shell structure composed of nucleic acid, polycation and lipid, combines both advantages of polyplexes and lipoplex.	Limited delivery of nucleic acid due to its size, low potential capacity	Cancer immunotherapy, Biomedical application	Superior colloidal stability, reduced cytotoxicity, and extremely high gene transfection efficiency	31-33
Inorganic nanoparticles 	Stable nanocarriers with tunable size, shape, and surface functionalization.	Non-biodegradable, with possible long-term accumulation.	Imaging, and theranostics, photothermal/photodynamic therapy, drug/gene delivery.	Often show good loading or payload delivery efficiency. Toxicity can vary widely with material type, size, coating, and dose.	34,35

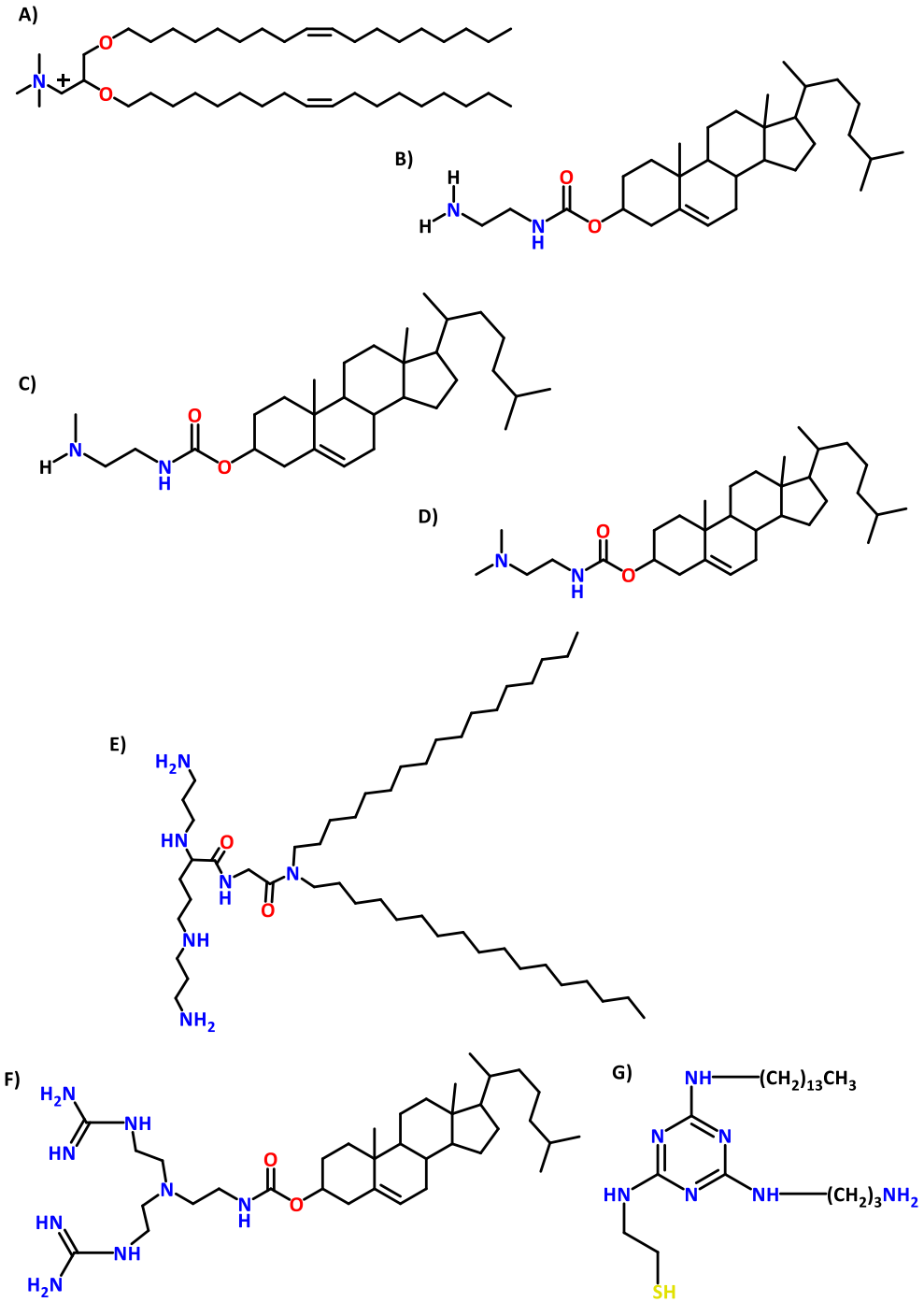


Figure 6. *Chemical structure of cationic lipids with different headgroup domains: A) DOTMA; B) AC-Chol; C) MC-Chol; D) DC-Chol; E) DOGS; F) BGTC; G) Triazine ring-based cationic lipid. Adapted with permission from ref 50. Copyright (2020) Elsevier B.V.*

(DOTMA), which demonstrated effective transfection however, the cytotoxicity remains a challenge. Subsequent generations of cationic lipids and lipid-based nanocarriers have been discovered to improve the delivery efficiency across different cell lines.[49] The representative structure of cationic lipid DOTMA and its linker bonds for cationic lipids are represented in **Figure 7**.[52]

In addition to DOTMA, Leventis and Silvius in 1990 have synthesized another lipid named as 1,2-bis-oleoyloxy-3-trimethylammonio propane (DOTAP). This DOTAP and DOTMA are structurally similar, but it differs only in the ester bonds rather than the ether bonds, which will link the oleoyl chains to the glycerol backbone, which also increases the biodegradability. However, no significant difference in transfection efficiency or cytotoxicity was observed between the synthesized DOTMA and DOTAP-based systems.[53] Further to improve the membrane fusion and nanoparticle stability, dioleylphosphatidylethanolamine (DOPE) and cholesterol, as well as PEGylation strategies, were used as a helper lipid, which increases the overall efficiency. Ju *et al.* have synthesized a cholesterol-based cationic lipid 3β-[N-(N', N'-dimethyl aminoethyl)-carbamoyl] Cholesterol (DC-Chol), which has been demonstrated effective transfection and has been used in liposomal formulations. Cholesterol has been used to improve the membrane fusion and stability owing to its hydrophobic domain.[54,55] To further improve the nucleic acid binding and delivery efficiency, the head group was modified with polyamines or basic amino acids. Moreover, the lipid stability and the gene transfer performance are significantly improved by the nature of the linker (ester, amide, ether or carbamate), while the ether-and amide-linked system

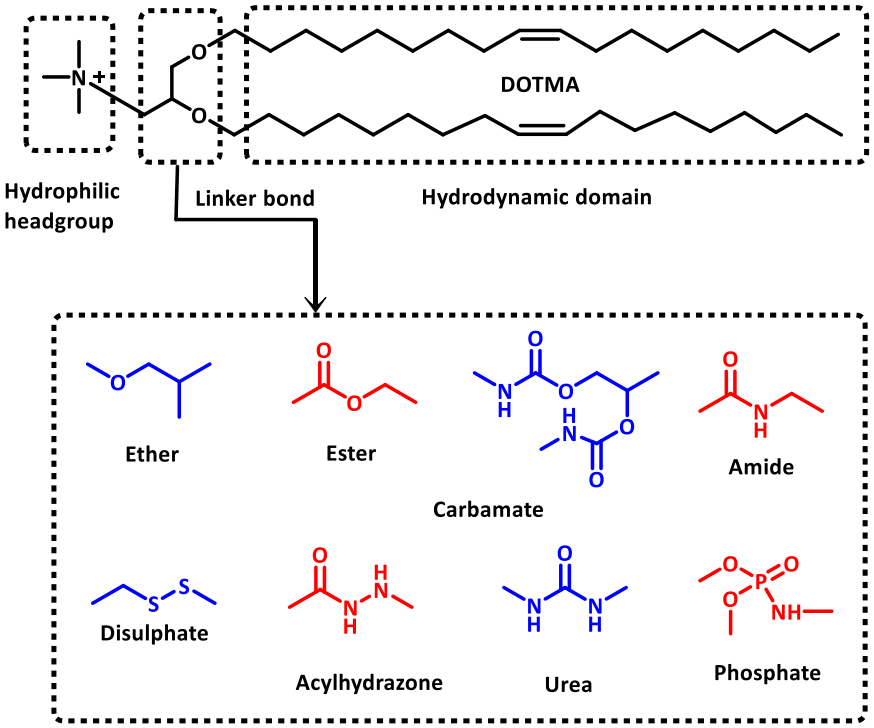
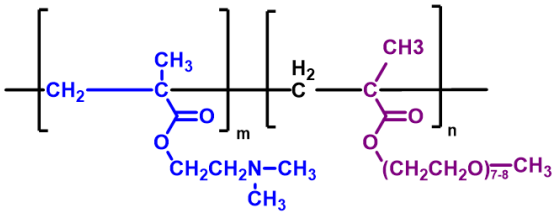


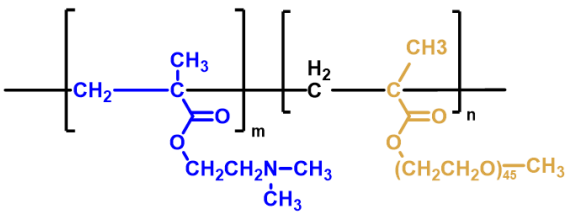
Figure 7. *Representative structure of cationic lipid DOTMA and its linker bonds for cationic lipids. Adapted with permission from ref 52. Copyright (2018) Elsevier B.V.*

generally exhibits improved stability and transfection efficiency.[56] Since then, numerous lipid-based and cationic lipid nanoparticles have been developed and investigated. The higher transfection efficiency for plasmid DNA was demonstrated by N', N'-dioctadecyl-N-4,8 diaza-10-aminodecanoyl glycine amide (DODAG), which was formed into cationic nano lipoplexes. Additionally, it was demonstrated that DODAG was more effective than the transfection reagent Lipofectamine 2000 at delivering plasmid DNA to OVCAR-3 and HeLa cell lines.[57] Further research is focused on developing the next generation cationic lipids with optimized head groups, linkers, and hydrophobic tails to improve the biocompatibility, reduce toxicity and achieve safe and efficient clinical transfection in gene therapy.

3.2. Cationic Polymers-Based Gene Delivery

Among various non-viral systems, cationic polymers or polyplexes are the most efficient candidate, as they have an easily modified chemical structure and high nucleic acid loading capacity to meet variety of gene delivery needs. These polymers carry a positive charge, which is protonated at the physiological pH.[58] These cationic polymers form a strong





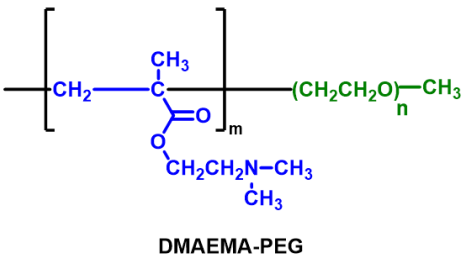
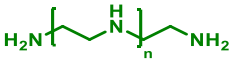
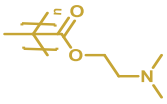
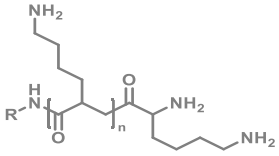
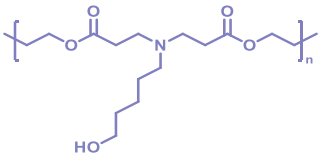
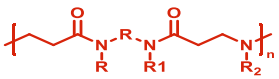
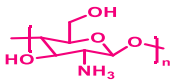
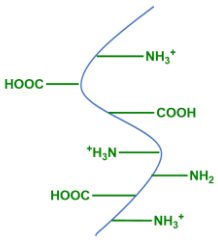
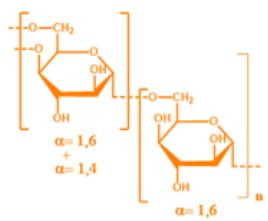
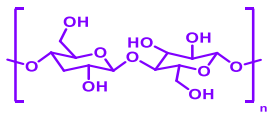


Figure 8. *Structure of PEG-based copolymers.*

electrostatic interaction to shield them from degradation, which leads to a polymer-gene complex, commonly referred to as polyplexes, which protects genetic materials from enzymatic degradation and facilitates cellular uptake via endocytosis.[59] Efficient gene delivery further requires endosomal escape, through the proton sponge effect exhibited by certain cationic vectors.

In Early 1965, Vaheri and Pagano investigated dextran functionalized diethylaminoethyl (DEAE) polymers as the first polymer for non-viral delivery systems.[60] Since then, a wide range of polymers with several modifications have been developed. Most of the polymers that are developed have a similar functional group which enables DNA condensation through electrostatic interactions. There are several types of cationic polymers, which are used as a gene carrier in which natural polymers such as chitosan, cyclodextrins, histone, gelatin, protamine, and synthetic polymers such as polyethyleneimine (PEI), PDMAEMA, Poly(propylenimine), poly(amidoamine) (PAMAM), Poly(L-lysine), and Poly(β-amino

Table 4. Cationic polymers: Structures, advantages, disadvantages and applications.

Cationic Polymer	Advantages	Disadvantages	Applications	References
(Polyethyleneimine) PEI 	Highly cationic, High efficiency, Efficient condensation and transfection.	Cytotoxicity, Non-degradable	Wound dressing, used in industrial, theranostic and biomedical applications.	63-65
Poly(dimethylaminoethyl methacrylate); PDMAEMA 	High cationic charge, Strong DNA binding, Efficient transfection, tunable molecular weight architecture.	Non-cytotoxic, Non-biodegradable, serum sensitive.	Water treatment, Coating and adhesives, personal care products, and drug/gene delivery applications.	61, 66-69
(Poly-L-lysine); PLL 	Biocompatible, biodegradable, water-soluble, and easy to functionalize, DNA compaction, and cell transfection.	Low transfection efficiency, limited endosomal escape, and cytotoxicity at higher charge densities.	Food additives, antimicrobial activity, agriculture and medical industries, and biomedical applications.	70,71
Poly(β-amino esters); PAE 	Biodegradable, biocompatable cationic polymer, pH-responsive (tertiary amines), stimuli-responsive release, tunable structure and charge	Narrow structure, serum sensitive, limited long-term stability, and reproducibility can be challenging.	On-demand drug delivery, disease prevention, tissue engineering biomedical applications antimicrobials and protein delivery.	72-74
Poly(allylamine); PAA 	Synthetic cationic polymer, water-soluble, Easy formation of polyplexes, Useful for both drug and gene delivery applications.	cytotoxicity at higher charge densities, limited biodegradability, and low transfection efficiency	Biosensing, antimicrobial coating, layer-by-layer films, functionalized micro/nanoparticles, Drug and Gene delivery applications.	75,76
Chitosan 	Naturally derived, biodegradable polysaccharide, low immunogenicity, good biocompatibility, low toxicity and controlled release and antimicrobial activity.	Low insolubility under physiological pH, Low transfection efficiency and Cationic under acidic conditions.	Water treatment, pharmaceutical, antimicrobial coating, wound healing, Gene/Drug delivery applications.	77-82
Gelatin 	Protein-based biopolymer, biodegradable, biocompatible, low immunogenicity, supports tunable gelation and matrix formation.	Limited mechanical strength, sensitive to temperature and pH, low cationic charge.	Food Industry, Cosmetics, food additive, pharmaceutical, gelling, film coating, scaffolds for tissue engineering, drug/gene delivery applications	83-86
Dextran 	Neutral, water-soluble polysaccharide, biocompatible, hydrophilic and easily modified.	Needs chemical modification, Low transfection efficiency.	Food additives, cosmetics, hydrogels, drug delivery and plasma expanders.	87-91
Cellulose 	Natural linear polysaccharide, biocompatible, hydrophilic, non-toxic, economic value and high mechanical properties, environmental friendly	Neutral backbone, Requires chemical modification, low transfection efficiency and limited colloidal stability.	Food additive, Wound dressings, tissue scaffolds, Paper packaging and drug and gene delivery applications.	92-94

esters) (PAEs).[61,62] The comparison table for multifunctional cationic polymer design are represented in **Table 4**.

Among the polymers, dextran, cellulose and gelatin are neutral polymers modified to introduce a cationic group for gene delivery. Among the polymers that are used as a gene carrier, PEI is considered the most efficient gene transfection system, which is considered the gold standard for gene delivery systems. Early in 1995, Behr *et al*.[95] has used PEI for the delivery of the oligonucleotides; their clinical application is limited due to their cytotoxicity, particularly at high molecular weights. To address this limitation, alternative polymers such as PLL and PDMAEMA have been explored, which show relatively low cytotoxicity and higher transfection efficiency.[95]

PDMAEMA, in particular, has received a lot of attention for its pH-responsive activity and ability to effectively condense nucleic acids. Hence, Rungsardthong *et al*. have investigated the copolymerization of PDMAEMA with Polyethylene glycol (PEG).[96] By incorporating PEG, the colloidal stability of the polymer-DNA complexes is stabilized, which leads to well-defined nanoscale polyplexes shown in **Figure 8**,[96] which prevents aggregation as well.

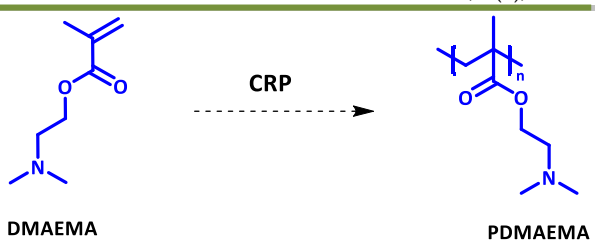


Figure 9. *Synthesis of PDMAEMA via RAFT polymerization.*

However, despite improving the stability and the DNA binding ability, PEG-containing copolymers often exhibit reduced transfection. The reduced transfection is due to the steric shielding effect of the PEG that limits cellular interaction and uptake. To further investigate the transfection efficiency of the PDMAEMA, the PDMAEMA-*co*-PEG was compared with the homopolymerization of PDMAEMA, in which the stability decreases and the transfection efficiency increases. This is

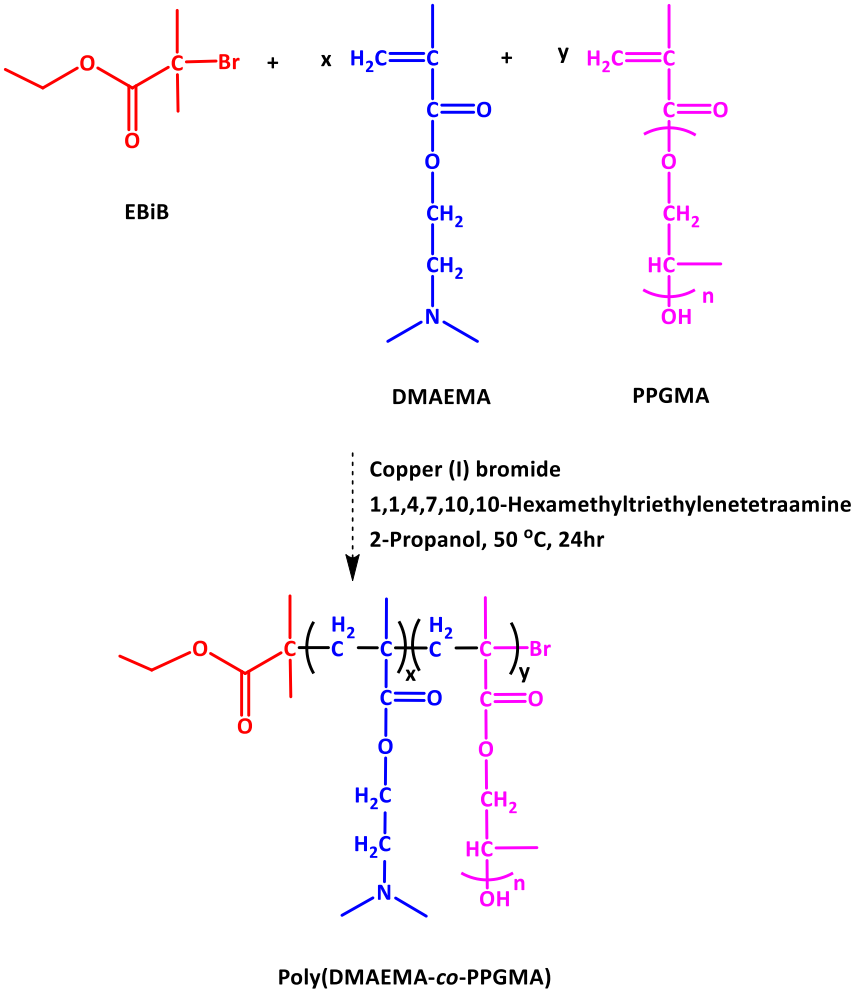


Figure 10. *Synthesis of poly(DMAEMA-co-PPGMA) using ATRP polymerization.*

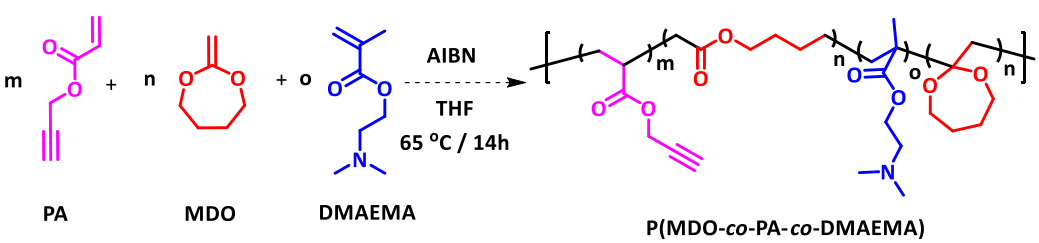


Figure 11. *Synthesis of poly(DMAEMA-co-PPGMA) using ATRP polymerization.*

due to its strong positive charge density, which promotes enhanced cellular uptake and efficient endosomal escape. Therefore, this study highlights the critical polyplex stability and gene delivery efficiency in the cationic polymer systems.[96] To achieve precise control over polymer architecture for (PDMAEMA), techniques such as controlled radical polymerization (CRP) are used, which are schematically represented in **Figure 9**.[96]

This technique is also referred to as reversible deactivation radical polymerization (RDRP). These can be applied to a extensive variety of monomers, enabling accurate control over the molecular weight, architecture, and end-group fidelity of vinyl polymers.[97,98] The three living radical polymerization methods that are gaining the most attention are the Reversible addition fragmentation chain transfer polymerization (RAFT),[99] Atom transfer radical polymerization (ATRP),[100] and nitroxide-mediated polymerization (NMP).[101] These polymerization techniques provide a well-defined polymers with controlled molecular weight, polydispersity and end group functionality, enabling for a well-defined polymeric gene carriers.

For example, Loh *et al.* has developed a cationic amphiphilic copolymer containing Poly(propylene glycol methacrylate) (PPGMA) as a hydrophobic segment and PDMAEMA as a cationic hydrophilic segment.[102] This PPGMA monomer has good temperature responsive behaviour, which can be soluble in lower temperature and the temperature increases when it becomes hydrophobic which leads to micelle formation. In this study, cationic micelles were synthesized via ATRP, which is a CRP which enables the preparation of new copolymers with precisely controlled molecular weight and low dispersity (Mw/Mn < 1.1), composition (block, graft, alternating gradient copolymers), and diverse functionalities.[103] ATRP is a catalytic process mediated by many redox-active transition metal complexes (Cu in CuI/L and X-CuII/L is the widely used transition metal).[104] The schematic representation of the synthesis of poly(DMAEMA-*co*-PPGMA) is demonstrated in **Figure 10**.[102] Further, the polymer showed relatively lower toxicity than the PEI (25 KDa). This synthesized polymer is compared with the PDMAEMA homopolymer as well, which shows relatively low cytotoxicity and better efficiency then other PDMAEMA-based polymers. The encapsulation of the paclitaxel further increased the efficiency of the copolymer. Nevertheless, the performance of the material was found to be similar to that of the PEI-based carriers.[102]

To further address the limitations, such as transfection efficiency and non-biodegradable nature of the PDMAEMA-based polymers, terpolymers like poly(MDO-*co*-PA-*co*-DMAEMA), were developed by Maji *et al*. using RROP reaction. The monomers used in the reaction were 2-methylene-1,3-dioxepane (MDO), propargyl acrylate (PA) and DMAEMA.[105] Further, the synthesized terpolymer was modified with PEG by alkyl azo click chemistry. The synthetic scheme for the terpolymer is represented **in Figure 11**.[105]

Significantly, these systems showed 15–20 times less toxicity at equivalent charge densities than polyethyleneimine (PEI), which demonstrated their improved biocompatibility. Due to partial DNA condensation and PEG's steric shielding effect, which restricts cellular uptake, the polymers' transfection effectiveness remained relatively low despite their ability to condense DNA into nanosized polyplexes. Cellular internalization and gene expression were considerably enhanced by the addition of cell-penetrating peptides like TAT.[105] Even though it has certain advantages, the system becomes more complex, and it is difficult to balance the biocompatibility, stability and transfection efficiency in polymeric gene delivery systems.

To further improve the biocompatibility and reduce the toxicity, Zhang *et al*.[106] has focused on developing a degradable polymer system. In this study, the copolymerization of DMAEMA with 5,6-benzo-2-methylene-1,3-dioxepane (BMDO) using a poly(ethylene oxide) (PEO)-based azo macroinitiator, as illustrated in **Figure 12**.[106] The incorporation of the BMDO introduces hydrolysable ester linkages into the polymer backbone, which gives controlled degradability. The resulting copolymers were further modified to quaternize using ethyl bromide (EtBr) to enhance their charge density and improve nucleic acid binding ability.

These degradable copolymers exhibit significantly reduced cytotoxicity relative to the gold-standard PEI (25 kDa), with IC_{50} values up to ~22-fold higher, indicating improved polymer structure, degradability, and charge, thereby improving the efficiency of the gene-delivery carrier.[106]

Earlier research primarily focused on hydrophilic/hydrophobic cationic polymers as gene carriers; however, the current trend has gradually shifted toward lipid-conjugated cationic polymer systems. Incorporation of hydrophobic moieties into these cationic polymers has been shown to reduce their toxicity. This is due to the increased hydrophobic segments, and improves the pDNA binding/loading affinity with efficient nanoplex stability, which facilitates the formation of a hydrophobic core-hydrophilic shell. When the cationic lipids are incorporated into the polymer, there will be an in membrane fusion and cellular uptake, which increases the gene transfection.[107,108]

By considering these advantages, Wang *et al*. have synthesized a natural steroid-based cationic polymer to enhance biocompatibility and maintain efficient gene transfection. In this study, steroid-based cationic random copolymers such as PMA6Chol-*r*-PDMAEMA (Chol-P1, P2, P3) and PMA6Dios-*r*-PDMAEMA (Dios-P1, P2, P3) were synthesized *via* RAFT with DMAEMA as a cationic hydrophilic monomer, as represented in **Figure 13**.[109] RAFT polymers are synthesized using chain transfer agents (CTAs), which are thiocarbonyl-containing organic compounds that enable controlled polymerization of a wide range of vinyl monomers. By using a suitable raft agent, it is possible to synthesize a new polymeric architecture with low polydispersity and various end-group functionalities. In this study, 2-(dodecylthio carbonothioylthio)-2-methylpropionic acid (DDMAT) was synthesized using a previous literature, which exhibited precise control over the polymer chain, which affects the toxicity and cellular uptake. As this study is developed by a steroid-based carrier, the cytotoxicity of the polymer decreased with increased hydrophobic segment, maintaining cell viability around 80%. This reduction in toxicity is due to the formation of larger nanoparticles, which will decrease the electrostatic interactions with negatively charged cell membranes. This research indicates that the hydrophobic and hydrophilic segments with the nature of the moiety, play a major role in cytotoxicity.[109] Hence, incorporation of appropriate hydrophobic steroid groups can enhance biocompatibility, making the system promising for safer gene delivery applications.

As the hydrophobic moieties decreased the toxicity, the incorporation of functional hydrophobic moieties which is emerging as a promising approach for next-generation gene delivery systems. In this study, Mapfumo *et al*.[110] has used Lipoic acid as a degradable and functional hydrophobic moiety. This monomer in particular has gained attention due to its antioxidant properties and redox-responsive disulfide functionality. In this study, DMAEMA was copolymerized with lipoic acid methacrylate (LAMA), which combines the alkyl methacrylate, demonstrating enhanced transfection efficiency and low cytotoxicity. In this study, the researchers explored three combinations of monomer units, such as DMAEMA, lipoic acid methacrylate (LAMA) with various methacrylate systems such as n-butyl methacrylate (nBMA), ethyl methacrylate

(EMA) or methyl methacrylate (MMA). This polymerization reaction is carried out by the RAFT polymerization process using 4-cyano-4 [[(ethylthio)thioxomethyl]thio] pentanoic acid (CPAETC) as the chain transfer agent and 4,4′-azobis(4-

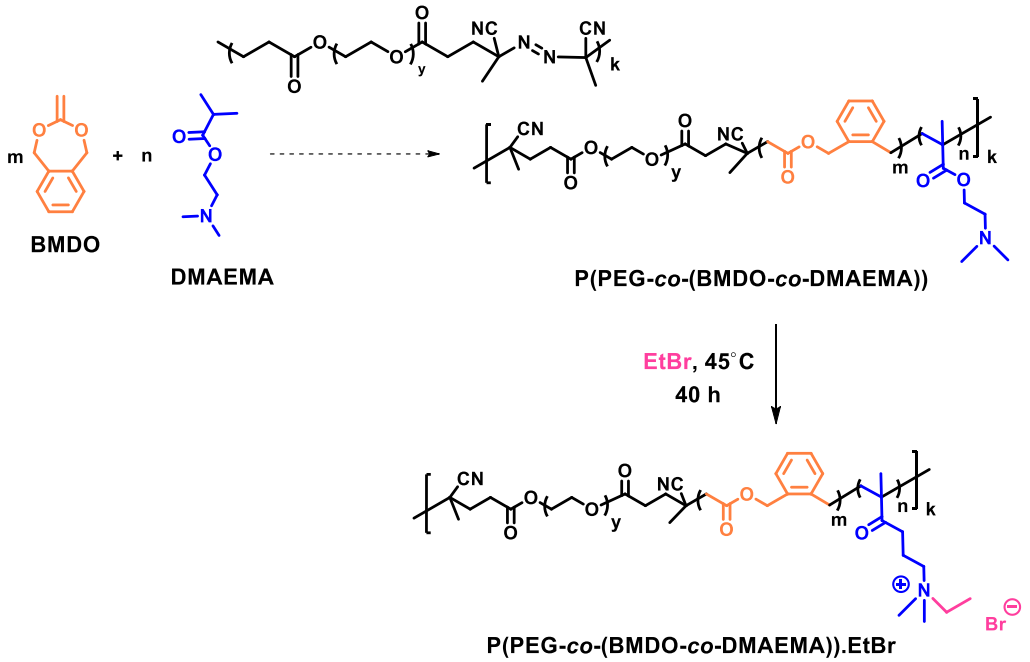


Figure 12. *Synthesis of poly(PEG-co-BMDO-co-DMAEMA) and poly(PEG-co-BMDO-co-DMAEMA).EtBr.*

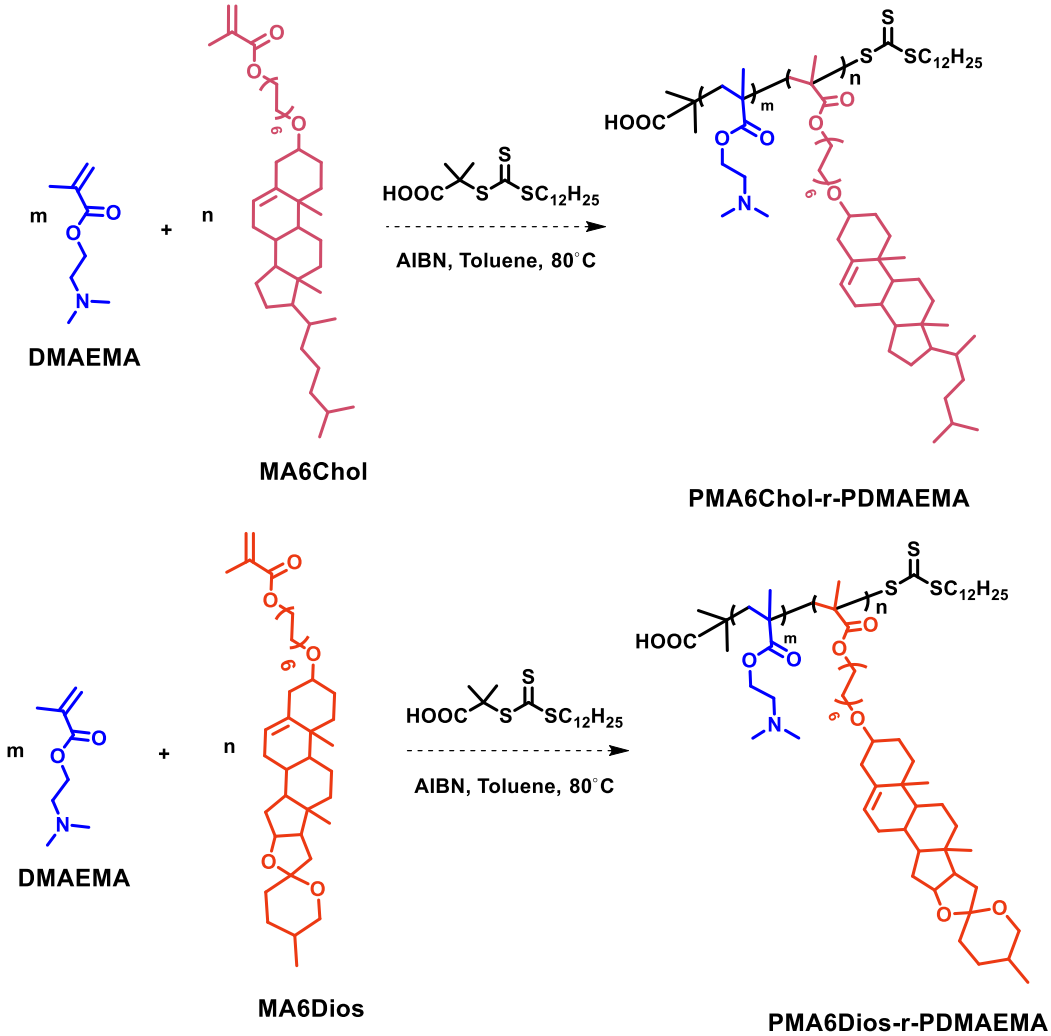


Figure 13. *Synthesis of (top) PMA6Chol-r-PDMAEMA and (bottom) PMA6Dios-r-PDMAEMA via RAFT polymerization.*

cyanovaleric acid) (ACVA) as the initiator. The synthetic scheme is represented in **Figure 14**.[110]

Copolymers based on DMAEMA and LAMA, combined with alkyl methacrylates, demonstrate enhanced transfection efficiency with low cytotoxicity. Among these, P(DMAEMA-*co*-LAMA-*co*-MMA) exhibits an optimal balance between cytotoxicity and transfection efficiency. However, increasing alkyl chain length leads to higher cytotoxicity, indicating that both the structure and functionality of hydrophobic segments play a crucial role in gene delivery.[110]

Further to enhance the performance and to improve the limited functional group the controlled living polymerization processes, can be modified by post polymerization techniques, which are primarily known as polymer analogous modification. This appealing strategy for the synthesis of functional polymers will produce new functional polymers from a reactive precursor.[111] These techniques are represented in **Figure 15**,[112] which includes active ester reactions, Michael-type additions, thiol exchange, Huisgen 1,3-dipolar cycloaddition (click reaction) and pd-catalysed coupling reactions. These induce additional functional groups onto the polymer backbone. These alterations enable the adjustment of polymer characteristics like charge, hydrophobicity and bioactivity, which makes them extremely valuable for uses such as drug or gene delivery systems.[112]

Overall, these studies show that the design of effective cationic polymer-based systems requires a proper charge density, stability, biodegradability, and functionality. The integration of stimuli-responsive and biologically active components represents a promising direction for the development of next-generation delivery vectors. Since cationic lipids and cationic polymers have been briefly discussed, a short introduction to lipopolyplex is provided, which is the next-generation lipid-polymer hybrid system for gene delivery applications.

3.3. Lipopolyplex-Mediated Gene Delivery

After the development of the first-generation cationic liposome-DNA complex, Lipopolyplex, emerged as the second-generation non-viral vectors, integrating cationic liposomes, polycations, and DNA in ternary form. The polyplex is made up of complex polynucleotide molecules with polycations, which are widely employed as nano carriers, while the lipopolyplex is made up of complex of polynucleotide molecules with cationic liposomes, which are used for nucleic acid delivery. Lipopolyplex, which combines the benefits of polyplex and lipoplex, has demonstrated exceptional colloidal stability, decreased cytotoxicity, and a very high gene transfection efficiency due to the synergy between lipid and polycation. [113] Based on the polycationic components that condensed the polynucleotide molecules, different kinds of LPPs are divided into different categories.[114] Their self-assembling nanoparticles can be made from a variety of ingredients, making them adaptable to a wide range of applications and possessing numerous functions.[115] Lipopolyplexes are made by using polycations to condense nucleic acids into rigid, homogenous particles (polyplexes), which are then encapsulated in cationic, anionic, or neutral liposomes. PLL, PEI, spermidine, spermine, protamine sulfate are the polycations that are frequently utilized in lipopolyplex systems. [116] The schematic representation of the Lipopolyplex for mRNA delivery is shown in **Figure 16**.[117]

However, despite their performance, conventional lipopolyplex systems rely on structurally simple polycations, which often limit precise control over physicochemical

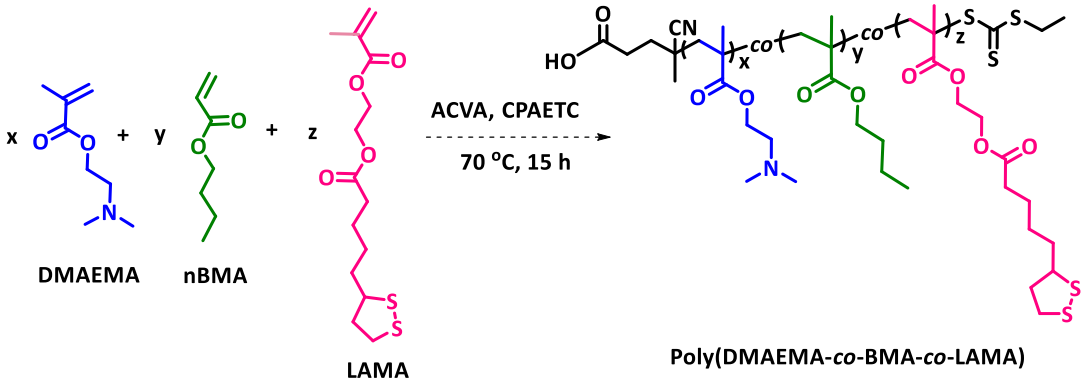


Figure 14. *Synthesis of poly(DMAEMA-co-BMA-co-LAMA) via RAFT polymerization.*

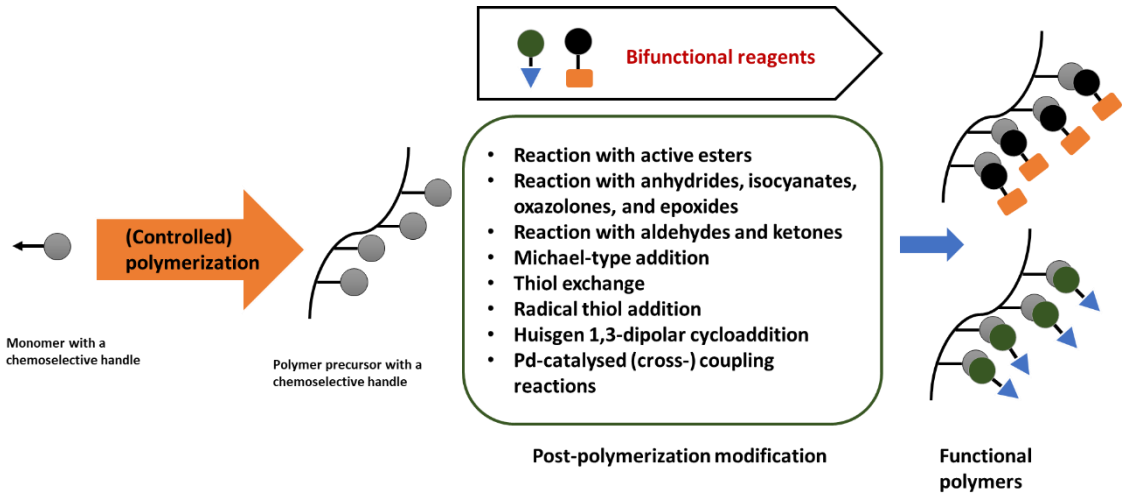


Figure 15. *Synthesis of polymer by post-polymerisation techniques.*

properties and intracellular delivery pathways. In this context, the development of advanced multifunctional cationic systems has emerged as a powerful system to overcome these limitations. By incorporating the multifunctional domains, such as cationic segments for efficient nucleic acid condensation, hydrophobic or lipid moieties for enhanced membrane interaction, and stimuli responsive groups for controlled intercellular release, these polymers enable improved nucleic acid interactions and delivery efficiency. These multifunctional polymer systems also provide biocompatibility, enhanced transfection efficiency, and greater control over gene delivery.

4. Recent Advances in Multifunctional Cationic Systems for Gene Delivery Applications

The synthesis methodologies for multifunctional cationic systems are explained, in which Hui Yun *et al*. initiated the synthesis of peptide-lipoic acid monomers using Solid-phase peptide synthesis (SPPS) on Wang resin. The researchers have designed three pentapeptide sequences, namely the -Lys-Leu-Gly-Gly-Gly, Lys-Leu-Lys-Gly-Gly, and Lys-Leu-Lys-Gly-Lys- to balance the charge, flexibility and structural stability for gene delivery efficiency.[118] The peptide-conjugated monomers are shown in **Figure 17**.[118]

After the successful completion of the synthesis of the peptide synthesis, lipoic acid was subsequently conjugated to the *N*-terminus using the conventional HBTU/DIEA coupling method. This conjugation step introduced redox-active disulfide linkages, imparting redox sensitivity crucial for controlled intercellular gene release. Finally, the peptide-lipoic acid conjugates underwent anionic ring-opening polymerization, forming cationic polymers with controlled molecular weights and redox-sensitive linkages. These structural features enhance gene binding and facilitate controlled, redox-triggered gene release. Overall, this approach allowed the development of peptide-based, lipoic acid-functionalized monomers tailored for non-viral gene delivery applications.[118]

In a related approach towards enhancing non-viral gene delivery, Amarnath *et al.* has synthesized a cationic polyethyleneimine (PEI) based non-viral gene carrier for developing a series of PEI derivatives for efficient delivery of plasmid DNA (pDNA). This study specifically focuses on enhancing the transfection efficiency and biocompatibility of low molecular weight PEI (Mw ~1200 Da) through lipid modification.[108] In this synthesis, PEI was grafted with lipids such as lauric acid, oleic acid and linolenic acid via two different linkers, gallic acid (GA) and p-hydroxybenzoic acid (PHBA). The reaction undergoes EDC/NHS coupling in the presence of triethylamine (Et_3N), allowing multiple lipid molecules to attach to single linker sites on the PEI backbone. This process produced small lipid-PEI conjugates suitable for

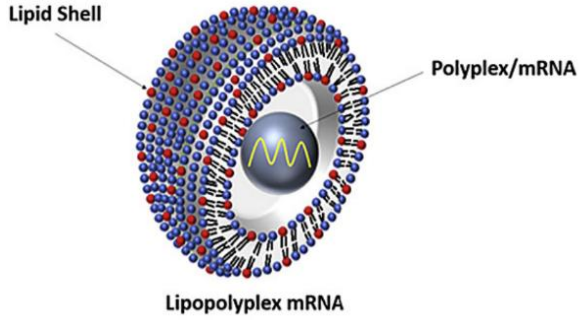


Figure 16. *Schematic representation of Lipopolyplex for mRNA delivery system. Adapted with permission from ref 117. (2017) Elsevier Ltd.*

evaluation. The Synthetic scheme is shown in **Figure 18**.[108] Finally, the resulting lipid-modified PEI derivatives were purified through precipitation and centrifugation to remove the unreacted PEI and lipid. These lipopolymers demonstrated effective pDNA complexation for gene delivery performance. These lipopolymers demonstrated effective pDNA complexation and transfection, highlighting their potential as non-viral carriers in gene therapy applications. Together, both studies focus on demonstrating the significance of combining cationic backbones with lipid or redox-responsive functionalities to achieve efficient, safe and controllable gene delivery systems. In which one system focuses on the peptide-lipoic acid conjugation for redox sensitivity and the other on the lipid-PEI grafting for membrane fusion and enhanced cellular uptake.[108]

Developing a safer and highly efficient gene delivery system is pivotal for the success of gene therapy, particularly in cancer treatment.[119] Previous strategies have primarily focused on modifying cationic polymers to improve their transfection efficiency, biocompatibility and stability, but they have used polyethyleneimine (PEI), which has been widely explored due to its transfection efficiency. Yet, polyethyleneimine (PEI) is considered limited by molecular weight-dependent cytotoxicity and instability in biological fluids.[120]

Nombeko Sikhosana *et al.* have presented the development and design of a high molecular weight hyaluronic acid (HA) based cationic amphiphilic nanosystem for plasmid DNA delivery.[121] In this study, pcDNA6.2-EmGFP is used as a reporter plasmid to assess transfection efficiency. They have used HA as the base polymer, which is anionic, has excellent biocompatibility and biodegradability, and is used in therapeutic delivery systems. HA has an affinity for CD44 receptors, which are expressed in cancer stem cells and damaged retinal pigment epithelial (RPE) cells, enabling receptor-mediated targeting. Despite the advantages, HA is an anionic polymer, so it cannot complex with the negatively charged nucleic acid. To overcome this limitation, HA was modified with 2-aminobenzimidazole (2AB). This modification reduced the overall negative charge density of HA, and it introduced cationic functionalities that facilitate electrostatic interaction with DNA. In addition to improved complex formation, the benzimidazole moiety may contribute to enhanced endosomal escape and cytosolic release through protonation-based mechanisms. The structural scheme is represented in **Figure 19**.[121]

After the 2AB conjugation, the HA backbone was further functionalized with oleylamine. The incorporation of this hydrophobic chain rendered the polymer amphiphilic and enabled self-assembly into nanoscale structures. The resulting HA-2AB oleylamine system forms stable nanoscale structures that combine with plasmid DNA via electrostatic and hydrophobic interactions. This amphiphilic polymer was intended to enhance DNA stability, bioavailability and retention at target sites. After the nanosystem's development, the polymer was evaluated in both HEK 293 and primary human RPE cells to assess its transfection efficiency across cellular environments. Various polymer-to-DNA ratios were optimised to examine complexation efficiency, cytocompatibility, and transfection outcomes, with GFP expression used as a reporter for gene delivery.[121] Overall, the HA-based cationic amphiphilic nanosystems demonstrated functional versatility across both permissive and challenging cell models, highlighting their potential for gene delivery in cancer and retinal therapies.

All the previous studies were primarily focused on improving polymer structure, to enhance transfection, stability and biocompatibility, while Camilla Pegoraro *et al*. extend these principles into multifunctional targeted nanoconjugates that integrate DNA binding, redox responsiveness,

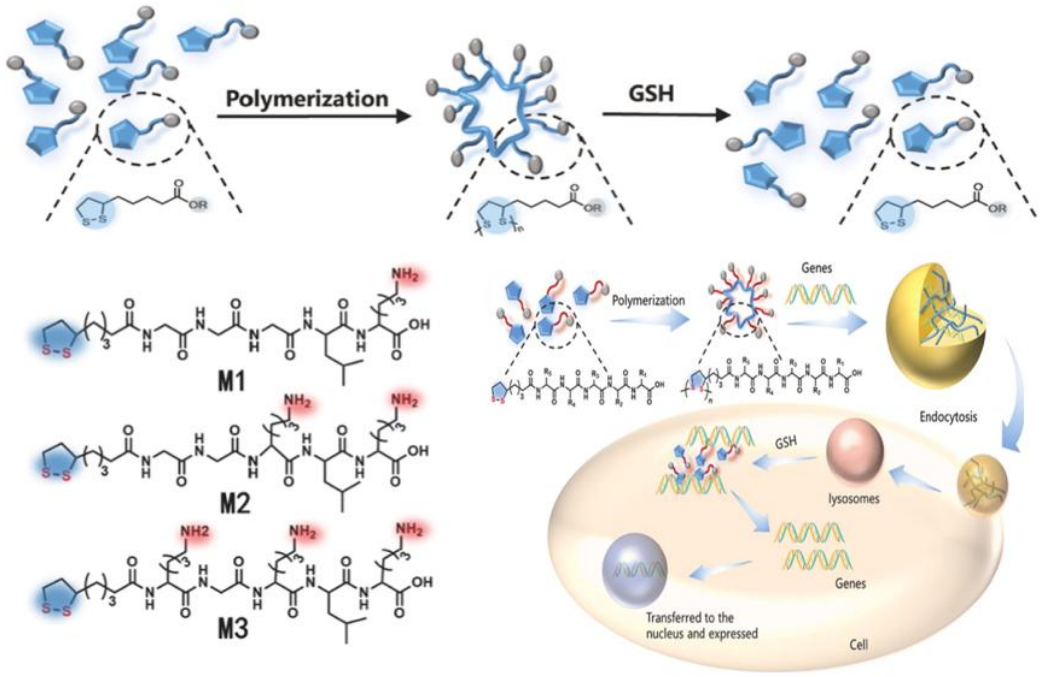


Figure 17. *Schematic representation of the nanoaggregates through the self-assembly process with the structure of the three monomers of lipoic acid-peptide and below Lipoic acid-peptide polymerization schematized. Adapted with permission from ref 118. Copyright (2024) American Chemical Society.*

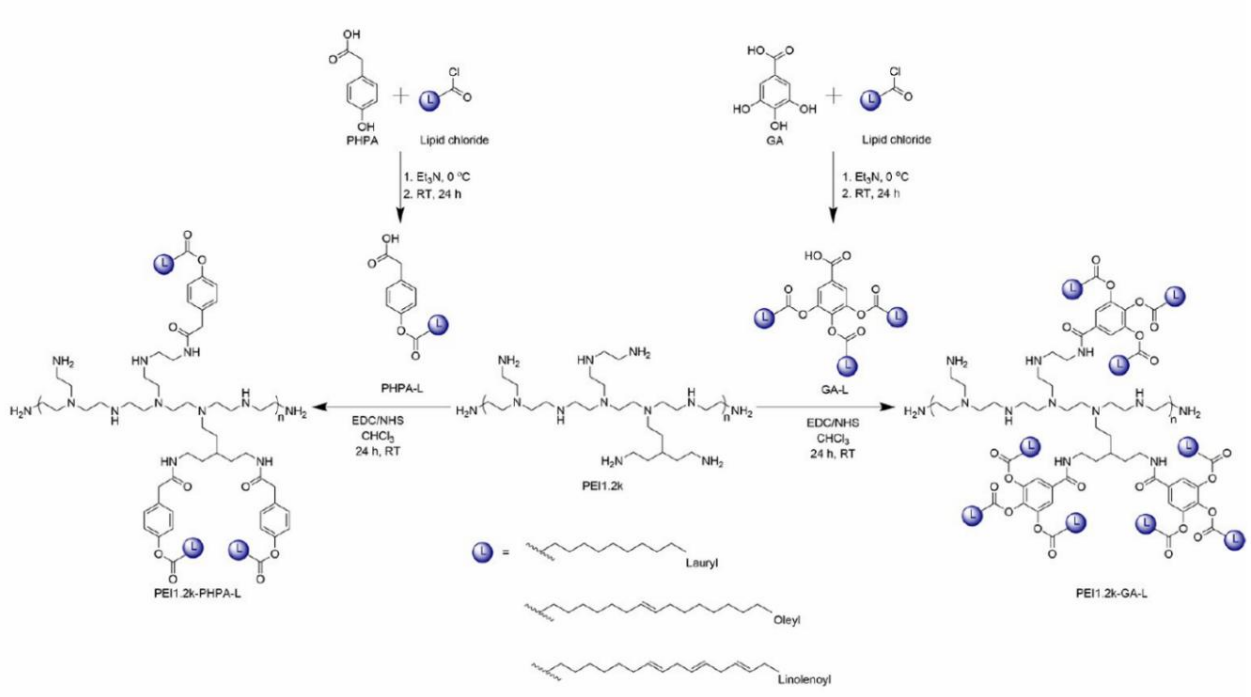


Figure 18. *Synthetic scheme for lipid-modified polyethyleneimine derivatives. Adapted with permission from ref 108. Copyright (2023) American Chemical Society.*

fluorescence tracking, and mitochondrial targeting for enhanced gene delivery.[122] **Figure 20** represents the multifunctional polypeptide-based nanoconjugates.[122]

The C-TRV3-A nanoconjugate is designed to improve the stability and facilitate mitochondrial targeting, which will increase the therapeutic efficacy. In this study, they have focused on synthesising a C-TRV3-A which works as a multifunctional gene delivery carrier.[122] The synthetic scheme is represented in **Figure 21**.[122] Initially, a trivalent molecule (TRV3) was prepared by sequential addition of the fluorescent dye (Cy5) and a mitochondrial targeting group (triphenylphosphonium, TPP) to a linker molecule, followed by introducing a redox-sensitive disulfide bond. Simultaneously, poly-L-ornithine, which is a cationic polypeptide, was PEGylated using mPEG-NHS, which will improve the water solubility and decrease toxicity. The TRV3 unit was then conjugated to the PEGylated poly-L-ornithine backbone via amide coupling. An additional dye (Atto 488) was added for tracking. The polyplex formed with an anionic polypeptide (VLC-A) will boost the transfection efficiency and reduce the toxicity. The synthesized nanocarrier, C-TRV3-A, combines

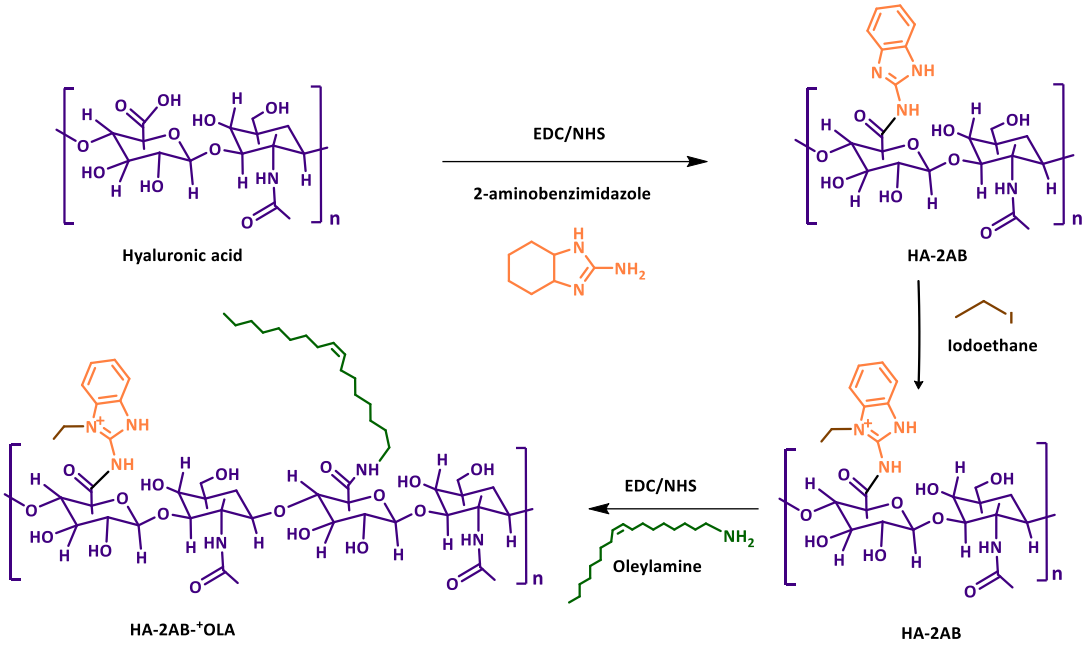


Figure 19. *Schematic representation for the synthesized cationic amphiphilic nanosystem (HA-2AB-+OLA).*

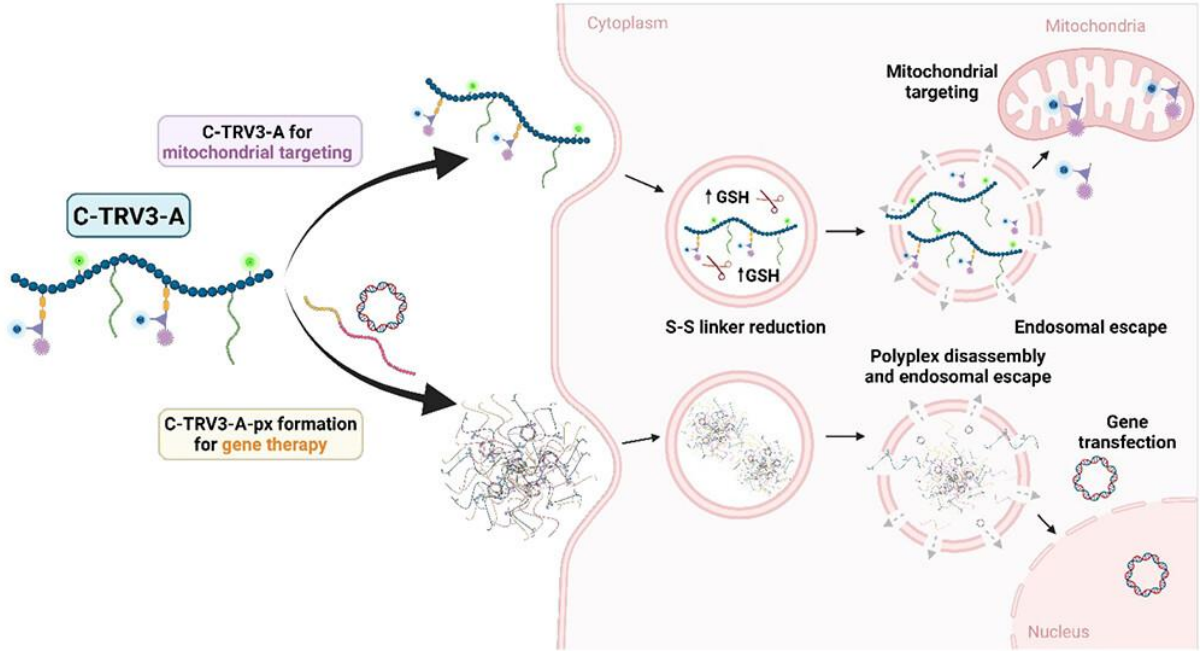


Figure 20. *Graphical representation of the multifunctional polypeptide-based nanoconjugates. Adapted with permission from ref 122. Copyright (2025) American Chemical Society.*

DNA-binding ability, mitochondrial targeting, fluorescence labelling, and redox-responsive release, making it a promising non-viral vector for gene delivery.[122]

By the development of multifunctional and targeted polymer-based carriers, Tabesh et al. focus on developing a gold nanostars (AuNs), which combine with a chitosan-cyclodextrin-poly(ethyleneimine) graft polymer.[123] The researcher has significantly focused on the synthesis of biocompatible gene delivery carrier, specifically the AuNs@CS-CD-bPEI (CCP) bio-nanocomposite. This synthesis procedure involves multi step process that integrates gold nanostars (AuNs) with a chitosan-cyclodextrin-poly(ethyleneimine) graft polymer.[123] The graphical representation is shown in **Figure 22**.[123]

This procedure involves the preparation of the AuNs with a few modifications.[124] A solution of $HAuCl_4$ is mixed with Triton X-100 and stirred at room temperature, followed by the addition of ascorbic acid and Au nanoseed suspension, which helps in the growth of the nano stars. The mixture is then centrifuged to isolate the AuNs, which are washed and suspended in Milli-Q water for further use. The next step involves the conjugation of chitosan (CS) with β-cyclodextrin (b-CD) and poly(ethylene imine) (bPEI).[125] The AuNS are then integrated with the CS-b-CD-bPEI polymer to form the AuNS@CS-CD-bPEI (CCP) BNC. This is achieved through a series of mixing and stirring processes, ensuring a uniform distribution of the components.

By combining gold nanostars with biocompatible polymers, this study aims to enhance transfection efficiency and provide a versatile platform for delivering genetic material in therapeutic applications. The successful synthesis and characterization of this nanocarrier pave the way for its potential use in various biomedical applications.[123]

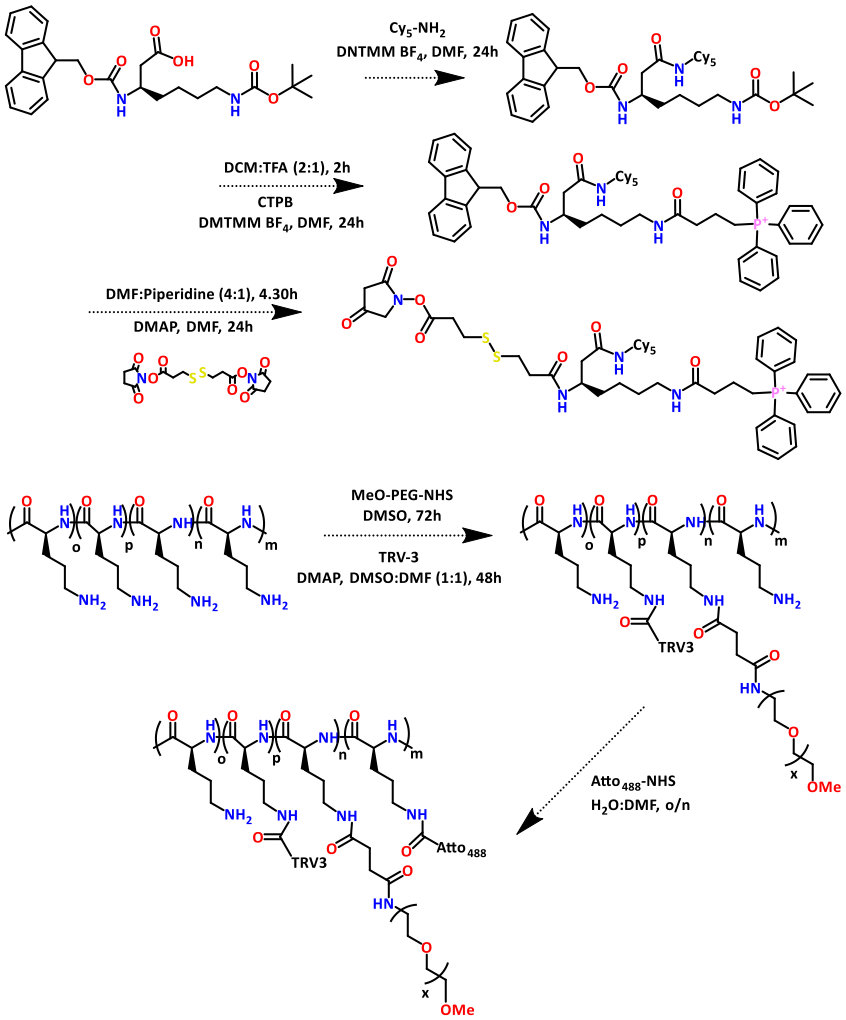


Figure 21. *Synthetic scheme for the preparation of the C-TRV3-A for the Multifunctional polypeptide-based nanoconjugates. Adapted with permission from ref 122. Copyright (2025) American Chemical Society.*

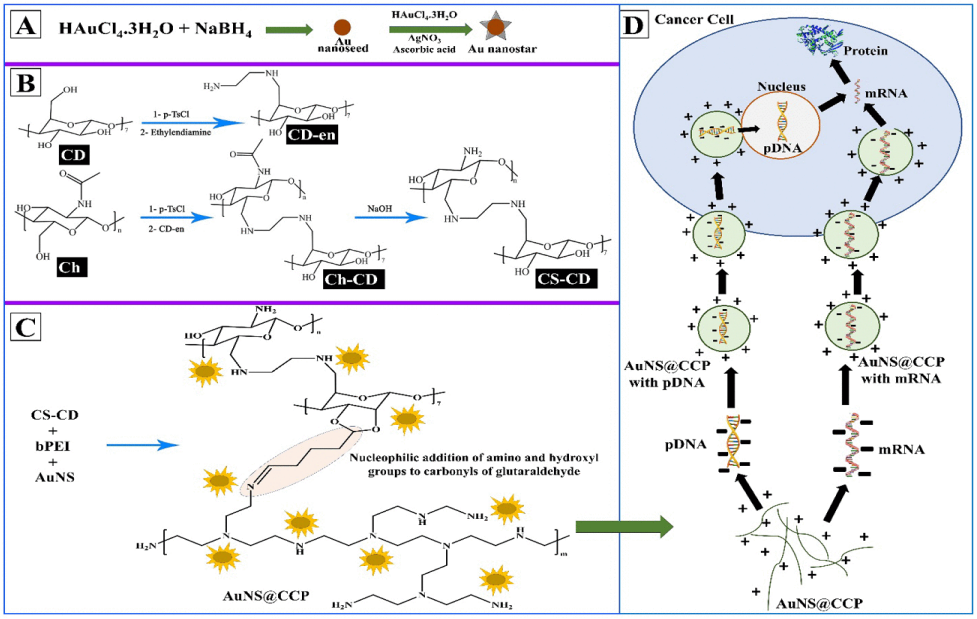


Figure 22. *Schematic representation of the preparation of AuNS@CCP and the mechanism for the transfer of nucleic acid into the cancer cells. a) Synthesis of Gold Nanostars (AuNSs) b) Chitosan–Cyclodextrin (CS–CD) Conjugate c) Preparation of AuNS@CCP Complex d) Transfection of nucleic acid into cancer cells. Reprinted from ref 123, published in 2024 by Royal Society of Chemistry, under the terms of the Creative Commons CC BY-NC license.*

The recent advancements have focused on creating biodegradable cationic polymers with enhanced gene delivery efficiency and reduced cytotoxicity. In this study, Luo *et al.* have synthesized a novel polymer, PDAB-CBA, which was designed with a dual-functional architecture combining a reducible, biocompatible backbone and a hydrogen-bonding

moiety to facilitate strong gene interaction while maintaining biodegradability.[126] The schematic representation is shown in **Figures 23**.[126]

The synthesis involves a sequential multistep process. Initially, p-aminobenzoic acid (PABA) and dicyandiamide (DCD) undergo a condensation reaction to form a pyrimidine-based intermediate (PD) containing both carboxylic and amino groups, enabling effective hydrogen bonding and gene interaction. This intermediate was then coupled with *N*-Boc-1,4-diaminobutane (Boc-DAB) through an amide coupling reaction between the carboxylic and amine groups. The Boc group was removed using trifluoroacetic acid (TFA) to develop an amine-functionalized intermediate PDAB. In the final step, PDAB is reacted with cysteine bisacrylamide (CBA) through a Michael addition reaction, where the primary amines of PDAB attack the acrylamide groups of CBA to form a linear poly(amidoamine) structure containing disulfide linkages. These linkages render the polymer reducible in intracellular environments, allowing for biodegradability and controlled gene release. The resulting PDAB-CBA polymer demonstrated improved transfection efficiency and low cytotoxicity, making it a promising candidate for safe and efficient non-viral delivery.[126]

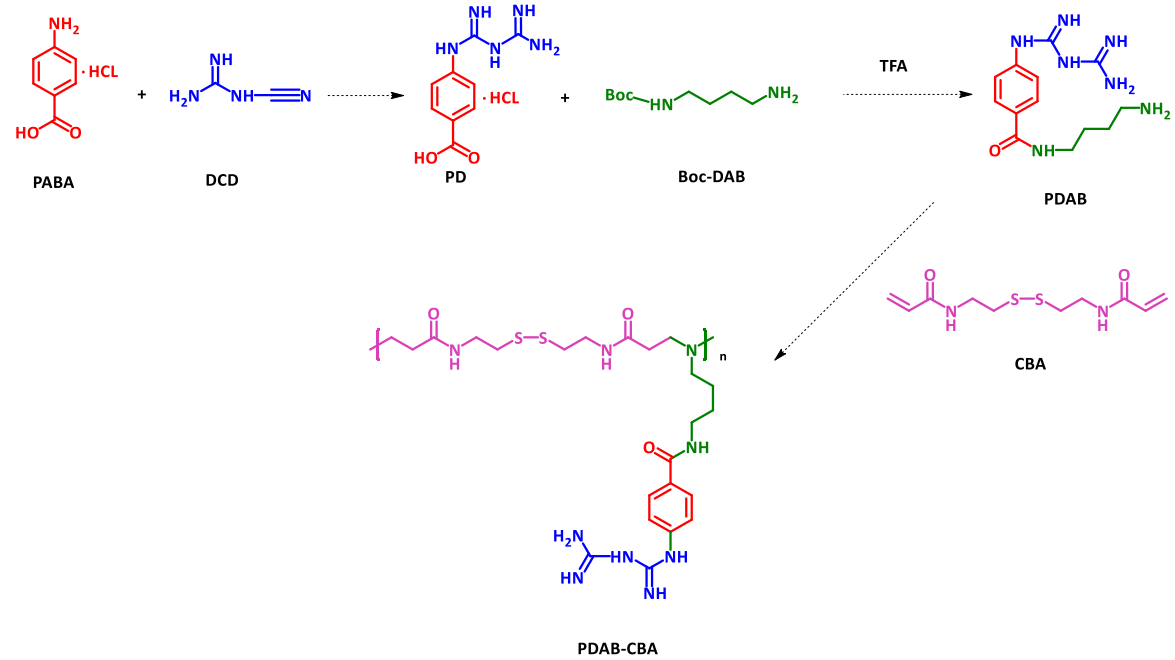


Figure 23. *Synthesis route of the linear polymer PDAB-CBA using Micheal addition reaction. Adapted with permission from ref 126. Copyright (2024) Elsevier B.V.*

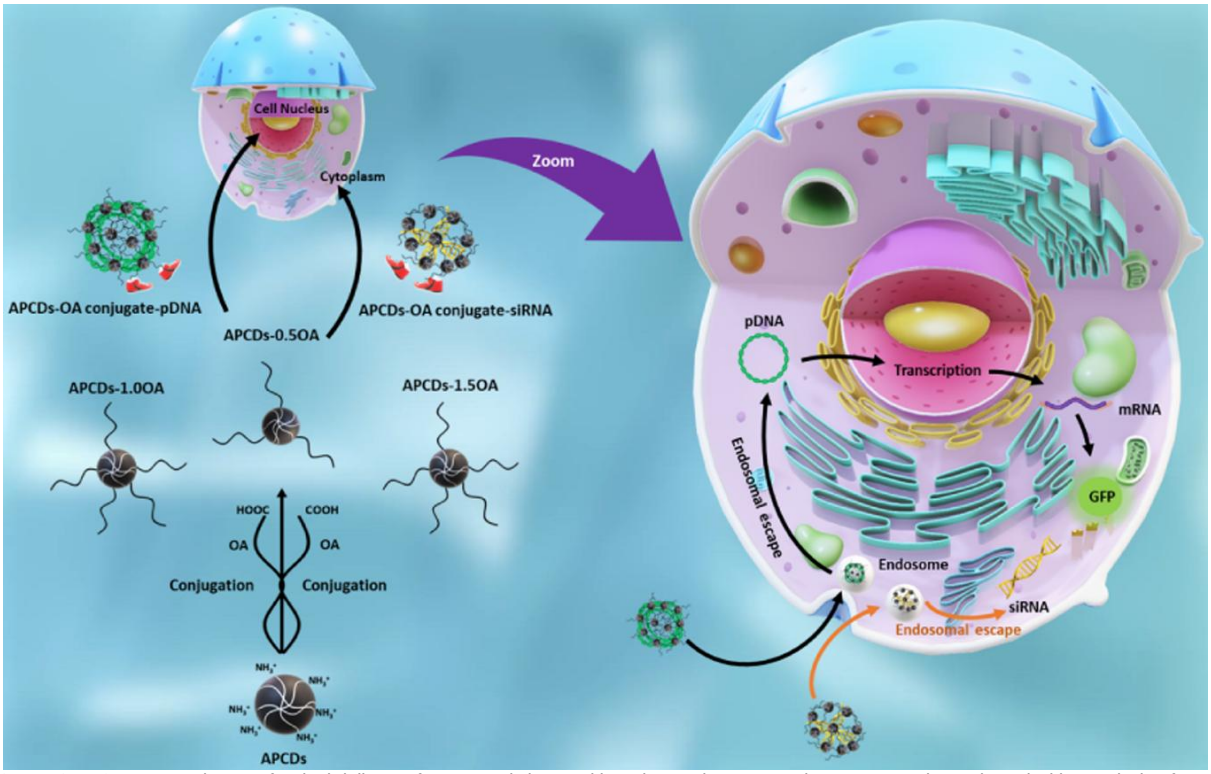


Figure 24. *APCDs-OA nanoconjugates for dual delivery of pDNA and siRNA with endosomal escape and gene expression. Adapted with permission from ref 127. Copyright (2024) American Chemical Society.*

To improve the gene delivery effectiveness, Chen *et al.* have investigated a hybrid nanocarrier technique by creating cationic carbon dots (CCDs) conjugated with oleic acid (OA), building on the developments made with biodegradable cationic polymers like PDAB-CBA.[127] The synthesis of the lipid-conjugated cationic carbon dots (APCDs-OA conjugates) involved a step synthesis, which includes the preparation of the APCDs, and their conjugation with oleic acid (OA). Initially, the APCDs were synthesized using a microwave-mediated approach. Arginine (Arg) and pentaethylenehexamine (PEHA) were used as precursors. To synthesise the APCDs, firstly, the arginine and pentaethylenehexamine were dissolved in deionised water (DI water). The dissolved solution was shaken vigorously overnight. The solution was then microwaved, followed by natural cooling to room temperature. The product obtained was dispersed in DI water for 20 minutes and ultrasonicated for 5 minutes. Further, ultracentrifugation was carried out using a sterile poly (ether sulfone) membrane, and dialysed against DI water for 3 days using a 100-500 Da molecular weight cut-off dialysis tubing. The obtained solution was lyophilized for 3 days to obtain APCD powders. Further, the prepared APCDs were conjugated with the OA through EDC/NHS-mediated amidation reactions. Three different mass ratios of APCDs and OA were used. The APCDs-OA with different concentrations were dissolved in ethanol. The solution containing the EDC in 0.5ml of water was added, and then the solution mixture was stirred for 30 minutes. Subsequently NHS solution was added and stirred continuously. This mixture was then combined with APCDs and was further agitated for 24 hours.[127] The graphical representation is shown in **Figure 24**.[127]

This study suggested that the conjugation of the CCDs with lipid structures like OA enhances gene transfection efficiency and improves cellular membrane interaction. This research works as a promising strategy for developing highly effective non-viral gene delivery vehicles that combine the advantages of both cationic carbon dots and lipid structures, paving the way for advancements in gene therapy applications.[127]

Further, the lipid conjugation was also investigated by Mittal et al. where researchers have synthesized a novel biocompatible delivery system based on cationic and cholesterol-containing polymers to address these limitations. The synthesis involves creating cationic lipid polymer hybrid carriers through a step ring-opening polymerization. They initiated by synthesizing MPA-AM-CBZ using 2-bromoethylamine hydrobromide and benzyl chloroformate, and then followed by they synthesized MTC-AM-CBZ monomer. Further polymerisation has been carried out as mPEG-b-p(MTC-AM-CBZ) polymerisation using $Sn(Oct)_2$ catalyst, conducted with palladium-catalysed deprotection to yield functional cationic polymers for therapeutic delivery. The synthesis creates two distinct polymer types: mPEG-*b*-p(MTC-AM) (cationic) and MPEG-b-p (MTC-chol) (cholesterol-containing) for formulating nanocomplexes that deliver C-Peptide and miRNA-29b therapeutically. The multi-step process is represented in **Figure 25**, which ensures biocompatibility, biodegradability and optimal electrostatic interactions for effective drug delivery.[128]

To overcome this cytotoxicity, Xin Bai *et al*. have developed a fluorinated poly(2-hydroxypropenimine) (PHP), which has a superior biocompatibility due to the presence of a hydroxyl group, which will improve hydrophilicity and reduce the inherent cytotoxicity.[129]

The synthetic scheme for the fluorinated PHPs is shown in **Figure 26**,[129] which was followed to synthesize a fluorinated poly(2-hydroxypropenimine) (PHP). In this, the fluorinated poly(2-hydroxypropenimine)s are synthesized using ethylene oxide ring-opening reaction. This reaction facilitates the incorporation of the fluorinated alkyl chains onto the PHP backbone, enhancing its properties for gene transfection. The study depicts how fluorinated epoxides are nucleophilically ring-opened onto a polyamine–polyol backbone to create fluorinated polyhydroxy polymers, PHP-FH4γ, PHP-FH8γ, and PHP-FH13γ. In this, the reactive hydroxyl and amine groups in the base polymer enable the attachment of epoxides with progressively higher fluorine contents (4, 8, and 13 fluorine

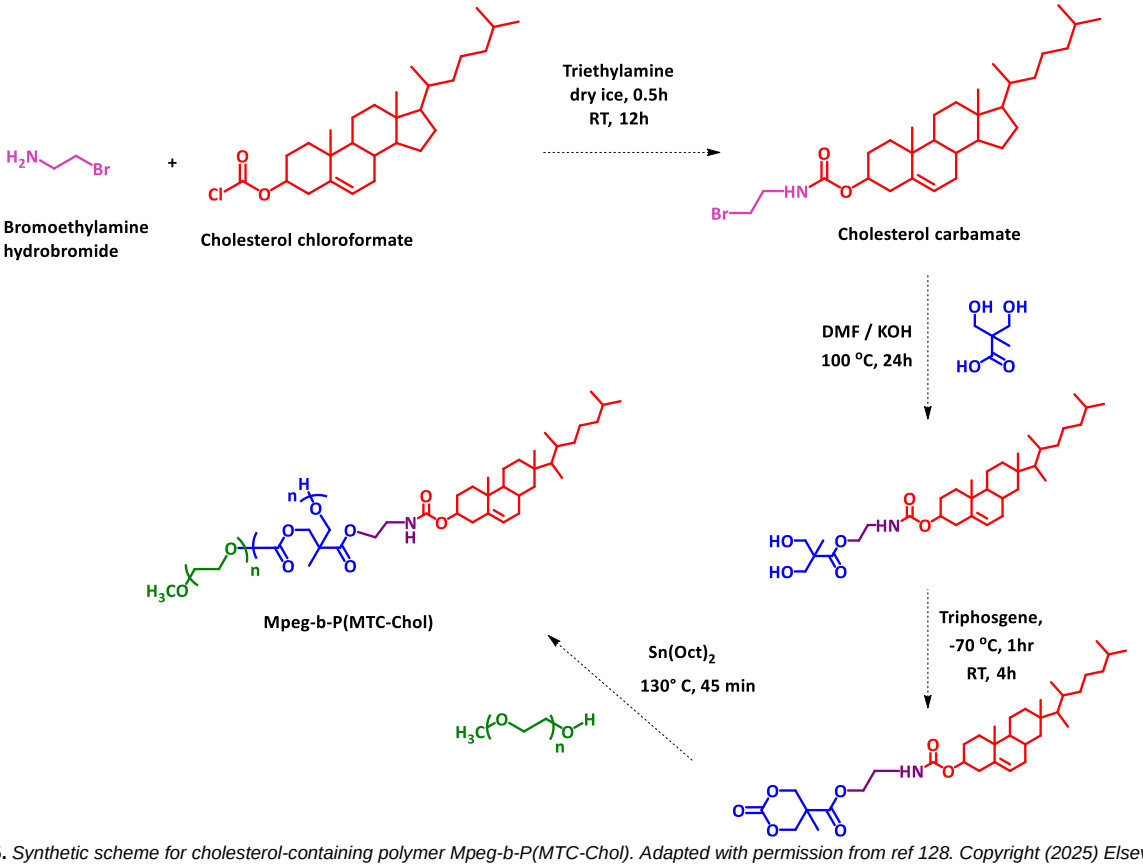


Figure 25. *Synthetic scheme for cholesterol-containing polymer Mpeg-b-P(MTC-Chol). Adapted with permission from ref 128. Copyright (2025) Elsevier B.V.*

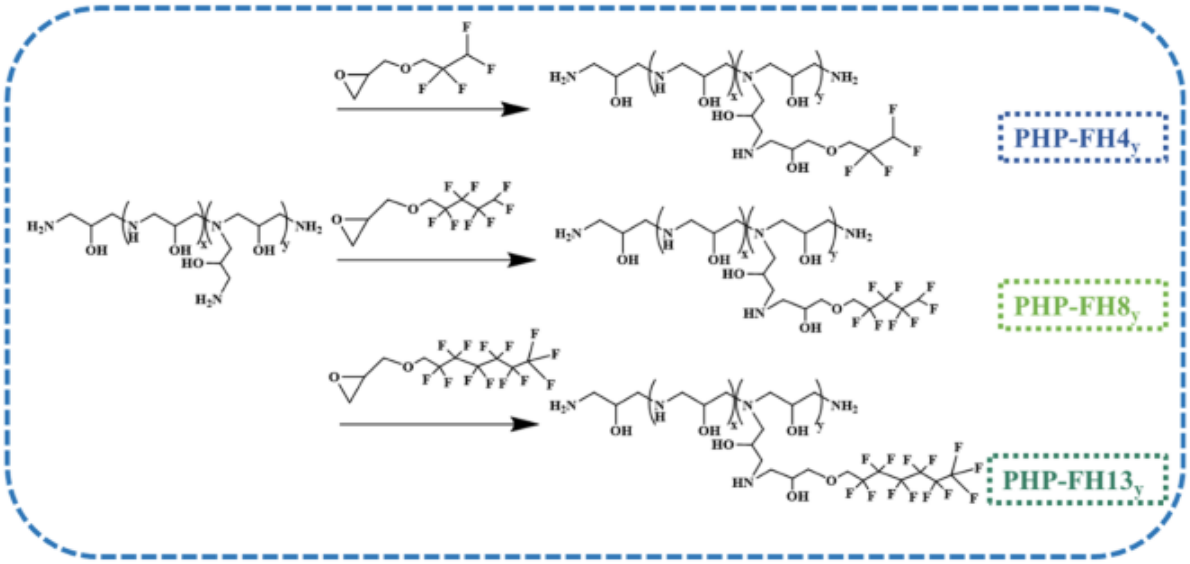


Figure 26. *Synthetic scheme for the fluorinated poly(2-hydroxypropenimine) polymers. Adapted with permission from ref 129. Copyright (2024) Elsevier B.V.*

atoms, respectively). These changes progressively improve the polymers hydrophobicity and membrane contact. The fluorinated derivatives are therefore well-suited due to their enhanced solubility, cellular absorption, and gene complex stability.[129]

A new approach of synthesizing a cationic gene delivery system has been developed and explored by Fielitz *et al.*[130] Here the researchers have synthesized a statistical copolymer using *N*-vinyl formamide (NVF) and *N*-vinyl pyrrolidone (NVP) using photoiniferter reversible addition fragmentation chain transfer polymerization (PI-RAFT) reaction. O-ethyl xanthate (Xan) is used as a CTA. The NVF repeating units are selectively hydrolysed with mild acidic conditions to yield Polyvinyl amine (PVAm) copolymers. The schematic is shown in **Figure 27**.[130]

The polymer's biocompatibility was evaluated, and the cytotoxicity was compared with the Linear PEI and lipofectamine 2000, which showed reduced cytotoxicity (23-to 1000-fold increase) and PEI (11-to 500-fold increase). The synthesized PVAm copolymers P75s demonstrated superior performance compared to the gold standard PEI, exhibiting significantly lower toxicity and high transfection efficiency. This study suggests that the developed and characterized PVAm copolymers have a high efficiency and less toxic gene delivery vectors, offering a promising alternative to current methods for various gene therapy applications. The above-mentioned study highlights the chemical modification to tailor polymer properties for enhanced gene delivery performance. These methods enable precise tuning of physicochemical and biological properties.[130] As we have explored a wide range of multifunctional cationic systems, several challenges have been observed, such as cytotoxicity, stability, cellular uptake, endosomal escape. These challenges and the strategies are discussed in detail in the following section.

5. Current Challenges and Strategies to Improve Gene Delivery

A wide range of cationic polymers, including dextran, PLL, PDMAEMA, PAMAM, dendrimers, PEI, PBAEs, and numerous other polymers, have been explored by researchers in the field of gene delivery vectors.[131] Stability, cytotoxicity, cellular uptake, endosomal escape, targeted delivery, and the effectiveness of gene transfection are some of the issues that stem from this process.[5] To overcome these challenges, specific strategies need to be developed,[132] such as modifications to the structure and the surface charge, alterations in the topological structure, modifications in the polymers, incorporation of the stimuli-responsive systems,

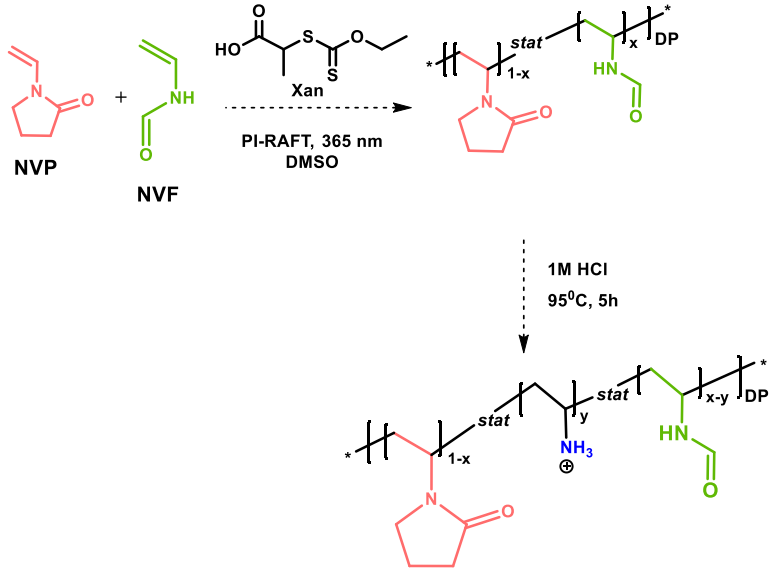


Figure 27. *Synthesis of PVAm using PI-RAFT polymerization of NVP and NVF with acid hydrolysis.*[130]

incorporation of machine learning, probing the influence of distribution of different molecular weight fractions, and the synergistic application of several approaches.[133] Excessive cationic carrier dosages cause cell membrane disruption, immunogenicity and inflammation, which leads to extreme cytotoxicity.[134] One efficient way to reduce the cytotoxicity is to use hydrophilic or anionic polymers to shield the charge of cationic polymers.[135] Cytotoxic effects should be reduced with minimal reduction in transfection efficacy by varying the ratio of cationic to anionic polymers. By removing the cationic component, we can reduce the surface charge.[136] Using various polymer topologies to create effective polymeric gene delivery vectors is encouraging. The switching of linear to branching architectures improves the polymers' assembly behaviour, adds a lot more functional end groups, and improves their interaction with DNA. Scientists can now more easily understand that the macromolecular structure has a major influence on their transfection efficacy. Thus, in order to investigate the impact of various topologies on gene transfection effectiveness, scientists have further constructed a variety of topological structures of polymers, including linear, branching, dendritic, star, and ring structures.[137] Polymeric vectors can have great transcription efficiency and excellent biocompatibility by rational polymer modification. Modifications such as lipid or fluoride conjugation into the polymers to address this issue and reduce the biotoxicity of the delivery systems. Fluorine modification in the polymers, such as PAMAM, PPI, peptide dendrimers, PEI, PBAE and PDMAEMA, is a useful technique to improve gene delivery. The carriers conformation hydrophobic interactions, lipophilicity, electronegativity, and basicity can also be controlled by fluorine substitution, which can change the carriers bioactivity and bioavailability as well as improve their membrane permeability and therapeutic benefits.[138]

Recent research in non-viral cationic systems has evolved beyond simple condensation of nucleic acids to more complex and multifunctional systems that enhance delivery by increasing selectivity in targeting, intracellular trafficking, and controlled release. In particular, ligand receptor targeting has emerged as an important strategy, where antibodies, peptides, sugars, folate, or hyaluronic acid bind selectively to receptors overexpressed on the surface of disease cells, thereby enhancing cellular uptake through receptor-mediated endocytosis. After internalization, these carriers can facilitate endosomal escape and improve cytoplasmic release of the genetic cargo, leading to higher transfection efficiency and reduced off-target effects. These endosomal escape is commonly achieved *via* the proton sponge effect, where buffering of endosomal pH induces osmotic swelling and membrane rupture, or through membrane destabilization by amphibilic/lipid modifified polymers, facilitating cytoplasmic release of the cargo. In addition to active targeting, significant attention has also been focused on using stimuli-responsive delivery systems. These systems remain stable during system circulation but release their payload in response to specific stimuli (*viz.* acidic pH, increased glutathione concentration, enzymatic activity, temperature, light, ultrasound, and/or magnetic fields)[139-141] as shown in **Figure 28**.[139]

Effective gene delivery to the tumour site is made possible by the cleaver stimulus-responsive delivery system, which reacts to intrinsic stimuli from the TME, such as high redox, acidic pH, and overexpressed enzymes.[142] Important cellular functions like motility, endocytosis and cytoskeletal remodelling are regulated by chemical and mechanical signals.[142,144] By enhancing intracellular transport and absorption, mechanical stimuli, including vibrations, shear stress and cyclic stretching, can improve gene transfer. This technique can enhance the transfection efficacy by up to 100 times while still being safe.[145] Modifying polymeric vectors with targeted ligands can improve their ability to accumulate at the desired site, thereby enhancing cellular uptake, controlled release, and overall transfection efficiency. Experiments can be done efficiently by machine learning, which forecasts polymer performance from sparse data. For instance, *Gong et al.* made accurate predictions for in vitro gene delivery by using random forest models to correlate PBAE polymer topologies with transfection effectiveness and toxicity. This method speeds up the polymer design and optimization.[146] Synthetic polymer vectors with both mixtures of high and low molecular weight components to protect DNA, shield cells from charges, and promote cellular absorption. In contrast to commercial reagents like jet PEI and Lipo3000, *Li et al.* achieved improved transfection efficiency by optimizing the polymer component. This highlights the importance of balancing polymer fractions

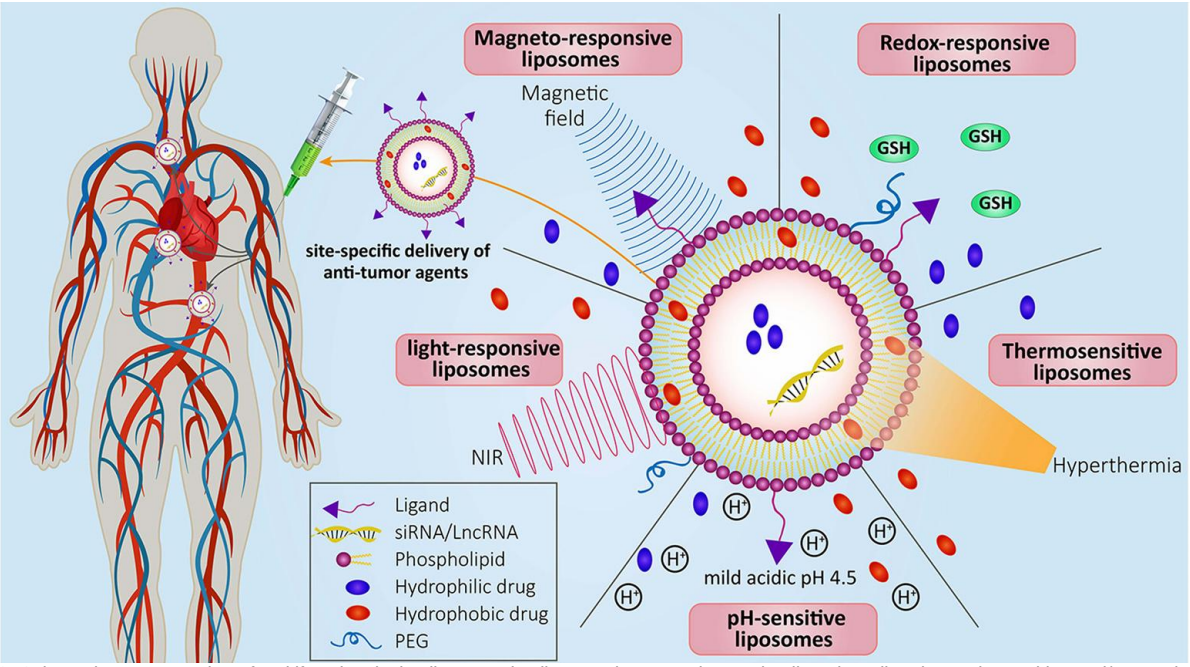


Figure 28. *Schematic representation of multifunctional stimuli-responsive liposomal systems integrating ligand-mediated targeting and internal/external stimuli for controlled gene delivery. Reprinted with permission from ref 139, published in 2024 by Taylor & Francis, under the terms of the Creative Commons CC BY license.*

for effective gene delivery.[147] In polymeric vector design, the drawbacks of individual techniques might be addressed by combining many strategies, such as creating a pH-responsive lipid-polymer nanoparticle that was combined with ultrasound-mediated microbubble destruction (UMMD) to improve CRISPR plasmid distribution and tumour penetration. This strategy achieved efficient gene inhibition and improved in vivo performance. The significance of using both active targeting and stimuli-responsive systems in conjunction with each other has emerged as a significant strategy for the design of next-generation cationic delivery systems. They allow for more controlled spatial and temporal control throughout the delivery process, thereby providing for greater safety and efficacy of the therapeutic delivery application.

In addition to the challenges in non-viral cationic systems, such as transfection efficiency, cytotoxicity, endosomal escape, cellular uptake, and targeted delivery, the development of multifunctional cationic systems faces a significant industrial barrier. The synthesis of complex architectures, such as star, dendritic, or block copolymers, often requires a multistep procedure and expensive monomers, which complicates the large-scale production and increases the manufacturing cost.[148,149] Reproducibility also remains as a major concern; a small change in the polymerization condition, will affect the change in the charge density, hydrophobicity and branching, which will affect the cytotoxicity, immunogenicity and efficiency upon scale-up. Synthesizing precise, positively charged, multifunctional polymers is more challenging, and requires a careful balance of scalability, yield and structural control, key for translation into bio applications.[150] Developing a simple controlled synthesis route for designing a cationic polymer is always critical for industrial-scale use. Future research should therefore focus on novel functionalities but also on simpler, scalable polymerization routes (*e.g.,* RAFT, ATRP) that enable well-defined, reproducible, and cost-effective cationic polymers compatible with good manufacturing practice (GMP) and regulatory requirements.[151,152]

6. Conclusion and Future Perspectives

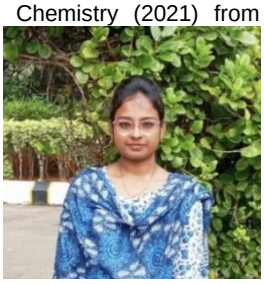
Non-viral cationic systems are designed to enable efficient and safe nucleic acid delivery. Although viral vectors often achieve high transduction efficiency, they are limited by safety concerns, high immunogenicity, and a complex production process. In contrast, the non-viral vectors are generally considered safer, less immunogenic, and easier to produce. To design these non-viral cationic systems, various polymerization methods and techniques are employed. This review focuses on synthesizing multifunctional cationic polymers, highlighting their key challenges (stability, transfection efficiency, toxicity, *etc.*) in achieving efficient and targeted delivery. To overcome these challenges, certain strategies (modifications to the structure and surface charge, alterations in the topological structure, modifications in the polymers, incorporation of the stimuli-responsive systems, *etc.*) are briefly outlined, emphasizing their potential to address current barriers in targeted gene delivery.

Future research should focus on safe and targeted nucleic acid delivery using cationic polymers, addressing key challenges such as immunogenicity, stability, toxicity, and efficiency. Emphasis on advanced architectures such as dendritic or hyperbranched designs with multiple head groups will enable tunable charge density, hydrophobicity, and site-specific release. Integrating stimulus-responsive elements (pH, redox, enzyme-sensitive) and degradable linkages (ester, acetal) promises controlled degradation and enhanced endosomal escape. These cationic polymers act as versatile platforms for gene/siRNA delivery, biosensors, and functional nanostructures. These polymers also provide potential for numerous applications such as coatings, drug delivery, biosensors, as well as delivery of genes and siRNA, with enhanced ability to escape endosomes and target the nucleus. As a clinical-transferable technology, the multifunctional cationic polymers must move from the preclinical phase toward commercial application in medicine. Several potential pathways could be developed to allow for local or systemic administration of gene or mRNA-based therapy, wound healing hydrogels, or implantable devices. The primary determinant of developing successful clinical applications of these polymers will depend upon their charge, density, biodegradability, and biocompatibility profiles. Before entering human trials, such systems would require rigorous evaluation of acute and chronic toxicity, immunogenicity, biodistribution, and clearance, particularly for long-circulating or non-biodegradable variants. In addition, manufacturing processes must be scalable and reproducible under GMP guidelines, ideally using modular or post-polymerization strategies to avoid complex, multi-step monomer syntheses. Finally, the design can be adapted to patient-specific needs-for example, by tuning cation–π crosslink density or side-chain functionality to match disease microenvironment or dosing requirements-thereby linking molecular innovation with real-world clinical impact.

Biographical Information

Ms. Sivaranjani Elumalai was born and raised in Chennai, Tamil Nadu, India. She received her Bachelor of Science in Chemistry (2022) from Anna Adarsh College for Women, Chennai. She subsequently completed her Master's degree in Chemistry (2024) from B. S. Abdur Rahman Crescent Institute of Science and Technology, Chennai. She is currently pursuing her Ph.D. under the guidance of Dr. Samarendra Maji at SRM Institute of Science and Technology, Kattankulathur Campus, Chennai. Her research focuses on the design and synthesis of lipid-based cationic polymers for gene delivery applications.

Ms. Lavanya Jujjuri was born and raised in Andhra Pradesh, India. She received her B.Sc. in Chemistry (2021) from Acharya Nagarjuna University, Guntur, Andhra Pradesh. She completed her Master's degree in Chemistry (2023) from GITAM School of Sciences, Visakhapatnam, Andhra Pradesh. She is currently pursuing her Ph.D. at SRM Institute of Science and Technology, Kattankulathur (KTR), Chennai under the guidance of Dr. Samarendra Maji. Her research focuses on the design and synthesis of degradable polymers for drug delivery application.



Mr. Anubhab Das was born and raised in Kakdwip, West Bengal, India. He received his B.Sc. in Chemistry (2017) from Dinabandhu Andrews College, Kolkata in West Bengal. He completed his Master's in Chemistry (2019) from Scottish Church College, Kolkata in West Bengal. He is currently pursuing his Ph.D. under the guidance of Dr. Samarendra Maji from SRM Institute of Science and Technology, Kattankulathur Campus, Chennai, Tamilnadu. His research has focused on the development of novel smart polymeric materials that may be utilized to identify nitrogenous contaminants in agricultural samples.

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Dr. Samarendra Maji obtained his M.Sc. degree in Chemistry (Organic) from Banaras Hindu University, Varanasi, in 2004 and obtained his M. Tech. degree in Materials Science and Engineering in 2006. He obtained a Ph. D. from Indian Institute of Technology, Kharagpur, under the supervision of Prof. Susanta Banerjee in 2010 and performed postdoctoral research with Prof. Andreas Greiner & Prof. Seema Agarwal (Philipps University Marburg, Germany) and Prof. Richard Hoogenboom (Ghent University, Belgium). Currently, he is working as Research Associate Professor, in the Department of Chemistry, SRM Institute of Science and Technology (SRMIST), Kattankulathur. His major research interests are Synthesis of polymers *via* CRP's (RAFT/MADIX), Stimuli responsive polymers & Polymeric-inorganic hybrid nanomaterials. He is the recipient of prestigious Alexander von Humboldt Fellowship of Germany (2010) and FWO Pegasus Marie Curie Fellowship of Belgium (2012).

Author Contribution Declaration & Information

Contribution

Sivaranjani Elumalai: Conceptualisation, Data curation, Writing – original draft. **Lavanya Jujjuri:** Data curation. **Anubhab Das:** Data curation. **Varsha K P:** Data curation. **Samarendra Maji:** Conceptualisation, Supervision, Writing – review & editing.

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Data Availability Declaration

There are no new data were created, hence data sharing is not applicable.

Declaration of Conflict of Interest

The authors declare no competing financial interest.

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