Recent advances on biocompatible and biodegradable nanoparticles as gene carriers

Article *in* Expert Opinion on Biological Therapy · March 2016

DOI: 10.1517/14712598.2016.1169269

6 authors, including:

Ahad Mokhtarzadeh
Tabriz University of Medical Sciences
48 PUBLICATIONS **174** CITATIONS
SEE PROFILE

Maryam Hashemi
Mashhad University of Medical Sciences
39 PUBLICATIONS **183** CITATIONS
SEE PROFILE

Mohammad Ramezani
Mashhad University of Medical Sciences
244 PUBLICATIONS **1,753** CITATIONS
SEE PROFILE

Expert Opinion on Biological Therapy

ISSN: 1471-2598 (Print) 1744-7682 (Online) Journal homepage: http://www.tandfonline.com/loi/iebt20

Recent advances on biocompatible and biodegradable nanoparticles as gene carriers

Ahad Mokhtarzadeh, Abbas Alibakhshi, Hajar Yaghoobi, Maryam Hashemi, Maryam Hejazi & Mohammad Ramezani

To cite this article: Ahad Mokhtarzadeh, Abbas Alibakhshi, Hajar Yaghoobi, Maryam Hashemi, Maryam Hejazi & Mohammad Ramezani (2016): Recent advances on biocompatible and biodegradable nanoparticles as gene carriers, Expert Opinion on Biological Therapy, DOI: 10.1517/14712598.2016.1169269

To link to this article: http://dx.doi.org/10.1517/14712598.2016.1169269

Accepted author version posted online: 21 Mar 2016.

Publisher: Taylor & Francis

Journal: *Expert Opinion on Biological Therapy*

DOI: 10.1517/14712598.2016.1169269

Recent advances on biocompatible and biodegradable nanoparticles as gene carriers

Ahad Mokhtarzadeh, [a,b,c] Abbas Alibakhshi [d], Hajar Yaghoobi [e], Maryam Hashemi [f], Maryam Hejazi [g], Mohammad Ramezani *[c,f]

[a] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran

[b] Department of Biotechnology, Higher Education Institute of Rab-Rashid, Tabriz, Iran

[c] Pharmaceutical Research Center, Department of Pharmaceutical Biotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[d] Department of biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[e] Department of biotechnology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[f] Nanotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[g] School of Medicine, Gonabad University of Medical Sciences, Gonabad, Iran

* Corresponding authors. Tel.: +98 513 7112470; Fax: +98 513 8823251. E-mail addresses: Ramezanim@mums.ac.ir (M. Ramezani)

Review

Recent advances on biocompatible and biodegradable nanoparticles as gene carriers

Ahad Mokhtarzadeh[a,b,c], Abbas Alibakhshi[d], Hajar Yaghoobi[e], Maryam Hashemi[f], Maryam Hejazi[g], Mohammad Ramezani*[c,f]

[a] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran

[b] Department of Biotechnology, Higher Education Institute of Rab-Rashid, Tabriz, Iran

[c] Pharmaceutical Research Center, Department of Pharmaceutical Biotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[d] Department of biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[e] Department of biotechnology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[f] Nanotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[g] School of Medicine, Gonabad University of Medical Sciences, Gonabad, Iran

* Corresponding authors. Tel.: +98 513 7112470; Fax: +98 513 8823251. E-mail addresses: Ramezanim@mums.ac.ir (M. Ramezani)

Abstract:

Introduction: Gene therapy mainly depends on the use of appropriate delivery vehicles with no induction of immune responses and toxicity. The limitations of viral gene carriers such as induction of immunogenicity, random integration in the genome of the host, limitations in the size, has led to a movement toward non-viral systems with much safer properties. Biodegradable and biocompatible polymeric nanocarriers due to several unique properties such as excellent biocompatibility, prolonged gene circulation time, prevented gene degradation, passive targeting by using the enhanced permeability and retention (EPR) effect, and possibility of modulating polymers structure to obtain desirable therapeutic efficacy, are among the most promising systems for gene delivery. However, biodegradable gene delivery systems have some limitations such as inadequate stability and slow release of therapeutics which have to be overcome. Thus, a variety of advanced functional biodegradable delivery systems with more efficient gene delivery activity has recently been introduced.

Areas covered: This review summarizes different aspects of biodegradable and biocompatible nano carriers including formulation, mechanism of intracellular uptake, various potential applications of biodegradable nanoparticles and finally recent studies on the therapeutic efficacy of these nanoparticles in sustained delivery of genes.

Expert opinion:

Biocompatible and biodegradable polymers will play a necessary and important role in developing new and safe carriers for oligonucleotide delivery. More working and the development of optimized polymers will reveal more their efficacy in the treatment of patients via helping in better gene therapy.

Key words: Gene therapy, Biodegradable, Biocompatible, Polymeric nanocarriers

Article highlights
• Biocompatible and biodegradable polymers play an essential and important role in developing new and safe carriers for oligonucleotide delivery.
• Nanoparticles based on polysaccharides represent excellent biocompatibility and are thus promising gene delivery carriers.
• Polyesthers such as PLA, PLGA, PCL and PHA in conjugation with cationic polymers show an important role in delivery of nucleic acid.
• Polyamide cationic nanoparticles due to biodegradability and non-toxicity are of good polymeric gene delivery vectors.
• Inorganic polyphosphates are almost ubiquitous polymers that have high potential in controlled release of genes after functionalization.

Expert Opinion on Biological Therapy

1 Introduction:

Gene therapy is a new paradigm and promising approach in medicine for the treatment of a variety of genetic diseases as well as an alternative method to conventional chemotherapy used in cancer therapy (1, 2). In general, "gene therapy" refers to the transfer of foreign DNA into cells to replace a missing or deficient gene or express, enhance or suppress a targeted gene. The most difficult challenges for the clinical application of gene therapy are the lack of safe, efficacious and controllable methods without eliciting adverse effects(1, 3). Therefore, the success of gene therapy largely depends on the choice of effective carriers that compact and

protect therapeutic genes from degradation by serum nucleases, extracellular enzymes and finally by intracellular degradation in endosomal/ lysosomal compartments (1, 4). Since gene therapy was first conceptualized in 1972, many different tools have been developed for efficient introduction of genes into target site as well as to overcome specific gene delivery barriers(5). Currently, there are different types of gene carriers being used for gene therapy applications and each has its advantages and limitations. These gene-transfer vehicles can be broadly classified into viral and non-viral subgroups. Early efforts in gene delivery have focused primarily on recombinant viral vectors such as retroviruses, lentiviruses, adenoviruses, adeno-associated viruses (AAV), and several other viral types (6, 7).Viruses can be used as gene carriers by removing pathogenic and un-necessary genes from virus genome and replacing it with a therapeutic nucleic acid. Such recombinant viral vectors will be nonpathogenic and will be able to infect and replicate in specific target cells. Despite the promise of viral vectors as efficient gene carriers, there are several obstacles including immunogenicity, toxicity, potential oncogenicity, random integration in genome of the host, restricted cell-targeting, storage difficulties, inflammatory potential, limitations in the size, and difficult scale-up procedures which limit their use as safe gene delivery vehicles(8). These limitations in viral gene carriers have given rise to the need for other delivery systems which mainly include non-viral systems with much safer properties, low cost, high flexibility and easier to manufacture than viral gene carriers(9, 10). Non-viral gene carriers are mostly based on nanoparticles (NPs), which are made from a variety of non-organic and organic materials such as polymers, lipids, peptides and their derivatives. Most of these NPs with net positive charge could effectively condense nucleic acids by electrostatic interaction and form particles with nano-scale sizes which could protect DNA from premature degradation by intra- and extracellular nucleases leading to modulation of gene

expression for a desired period of time (8, 11). Nevertheless, following the entry of positively charged NPs into the blood stream, interactions between the NPs surface and serum proteins occur which may lead to their removal by the mononuclear phagocyte system (MPS) in liver and spleen(12). On the other hand, accumulation of non-biodegradable NPs in the body, especially in liver and spleen,can lead to toxic effects(13, 14). Therefore, the need for biocompatible, biodegradable and non-toxic nanocarriers which could effectively condense nucleic acids and avoid accumulation inside the cells is evident (15). This review intends to summarize the current understanding on most important biocompatible and biodegradable NPs as gene carriers especially biodegradable polymers obtained from biological sources (Table 1).

Expert Opinion on Biological Therapy

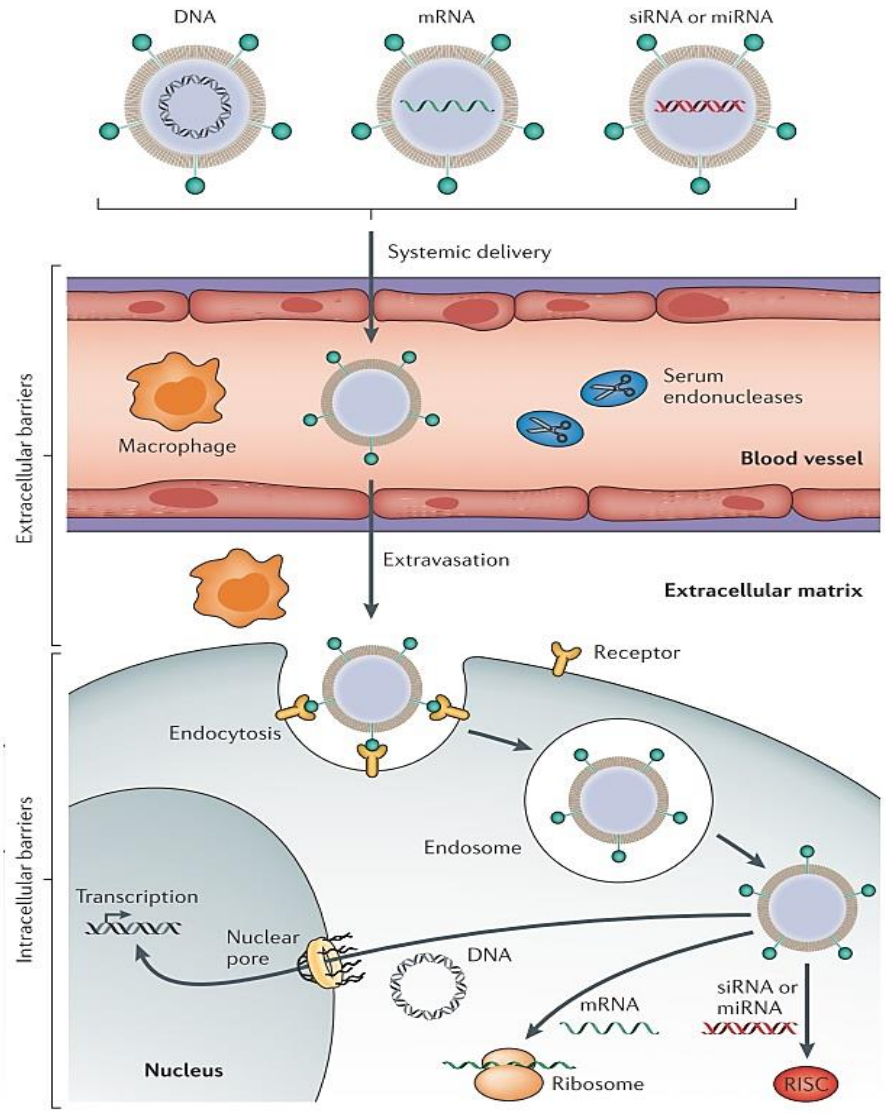

Figure 1. Barriers to successful in vivo delivery of nucleic acids using nonviral vectors.This figure was obtained with permission from reference(8).

Table 1.Biodegradable and biocompatible polymers in gene therapy

Polymer	Important examples	Chemical structure	Main resources	Advantages and disadvantages for use in gene delivery
Polysaccharides	Chitosan	Composed of randomly distributed β-linked D-glucosamine and N-acetyl-D-	Crustaceans; fungi	Biodegradability, biocompatibility, facile chemical modification on functional groups, diversity in

		glucosamine		polysaccharides structure, ability of receptor targeting (Hyaluronic acid), usually with low cytotoxicity
	Hyaluronic acid	β-(1-4)-linked repeating heteropolymer consisting of glucuronic acid and N-acetylglucosamine	Animal products	
	Dextran	α-(1,6)-linked homopolymerconsisting ofglucopyranose units with α-(1,2)/α-(1,3)/ α-(1,4)-glycosidic branch linkages	Bacteria	
Polyesters	Polylactic acid	Consisting of L- or D-lactic acid	Plant products such as corn starch; lactic bacteria	Biodegradability, biocompatibility, DNA-carrying capacity, the simplicity of large-scale production, long-term delivery, Lowcytotoxicity, the majority are generally hydrophobic and neutrally charged
	Poly (lactide-co-glycolide)	With different ratios between its constituent monomers, lactic (LA) and glycolic acid (GA)	Crude oil	
	Polycaprolactone	Ring opening polymerization of ε-caprolactone – a 2-Oxepanone homopolymer	Crude oil	
	Polyhydroxyalkanoates	Synthesized from hydroxyacids, HO-R-COOH	Bacteria	
Polyamides	Poly(ε-L-lysine)	Homopolypeptide of 25–30 L-lysine residues	Bacteria	Biodegradability, biocompatibility, to, formation of nanosized polyplexes with nucleic acids, Non-toxic
	Poly(γ-glutamate)	Repeating units of l-glutamic acid, d-glutamic acid or both	Bacteria	
Polyanhydrides	Polyphosphates	Consists of orthophosphate units (PO4) linking by high-energy phosphoanhydride bonds	All living cells	Biodegradability, biocompatibility, similarity to biomacromolecules and presence of pentavalent phosphorus in polyphosphates, Non-toxic, hydrophobic,

				functionalization is necessary before employing

2 What is biocompatibility and biodegradability?

Up to now, many different types of NPs such as carbon nanotubes (16), graphene oxide(17), dendrimers(18), liposomes (19), polypropylenimine(11), quantum dots (20), gold (21), silver and magnetic NPs (22) have been designed and introduced as gene and drug carriers (23-25). In order to be able to use nanomaterials in cancer therapy, it would be better to formulate therapeutic agents in safe, biocompatible and biodegradable nanoparticulate systems with lower overall toxicity in the body (26, 27). The selection of the NPs as gene carrier is dependent on different parameters such as 1) size and zeta potential of the desired NPs, 2) condensation efficiency 3) endosomal scape mechanism by which the polyplex released into the cytosol, 4) various surface modifications and functionality, 4) degree of biodegradability and biocompatibility of NPs (25, 28-30).

The word biocompatibility is referred to "the ability of a material to perform with an appropriate host response in a specific situation" (29, 31). In general, ahigh degree of biocompatibility of NPs is achieved when a NP interacts with the body without induction of undesirable effects such as toxicity, thrombogenicity, immunogenicity, and carcinogenicity. Therefore, non–biocompatible NPs may either stimulate immune system response or trigger inflammatory reactions which finally lead to faster removal by the immune system (32). The clearance of NPs is often a crucial step after the introduction of NPs into the body and determines their outcome. Size and surface charges of NPs are the most important factors in determining the NPs clearance rate. Many studies have shown that surface charge has the most important role in the fate of NPs,

generally, neutral or negatively charged NPs lead to less inflammatory reactions compared to positively charged NPs (29). Biocompatibility of nanomaterials depends on their structure, size, formulation and many otherfactors(29, 33). Therefore, we need appropriate and safe biodegradable nanoparticulate systems for the delivery of gene, drug, peptides, and proteins to be used in the field of nanomedicine as well as tissue engineering (29). Furthermore, degradation of these biodegradable NPs can be used as a mean of the release of the payloads (drug and gene) into the target cells (4). However, low gene transfer efficiency of most non-viral gene carriers must be improved by surface modification via appropriate chemical methods such as PEGylation for inhibition of undesired interactions with serum compartments as well as conjugation with targeting agents such as antibodies (34), peptides (2), aptamers(35) and other targeting ligands for receptor-mediated endocytosis. Nowadays, a variety of polymers, both synthetic and natural, have been introduced as biodegradable and biocompatible NPs for biomedical purposes.

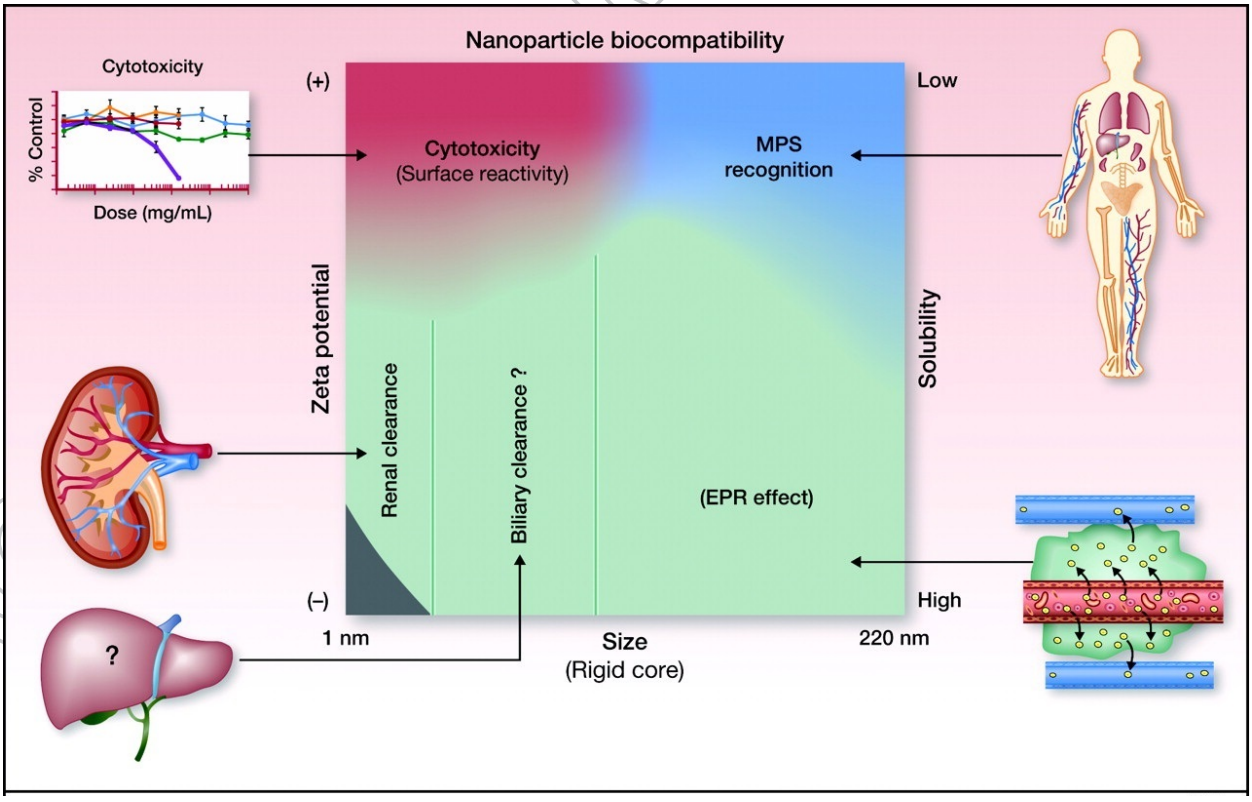

Figure 2. Nanoparticle biocompatibility trends. The zeta potential, size, and solubility affect the cytotoxicity (surface reactivity), clearance process (renal or biliary), MPS/RES recognition, and EPR effect.This figure was obtained with permission from reference (33).

3 Polysaccharides

Polysaccharide-based NPs because of their natural origin and unique physicochemical properties such as availability, excellent biocompatibility, biodegradability, low toxicity, highly chemical reactivity and low cost have attracted special interest in the fields of pharmacological and therapeutic applications. Naturally occurring polysaccharides are a large group of polymeric carbohydrates with long chains of monosaccharide repeating units adjoined by glycosidic linkages. Polysaccharides areabundant in nature obtained fromvarious sources such as algae (e.g. alginate), plants (e.g. pectin, cellulose, cyclodextrins), microorganisms (e.g. dextran, pullulan), and animals (chitosan, chondroitin, hyaluronic acid) (36). Structure and chemical composition of polysaccharides are often heterogeneous, neutral or charged, linear or branched with varying molecular weights (Mw). Polysaccharides have a larger number of functional groups(such as amines and carboxylic acids) in their glycosidic units which are prone to facile chemical modifications(37, 38). Structural modifications may impart better specificity for binding to the target site. Moreover, chemical modifications can improve the properties of the polysaccharides in such a way that could overcome their specific shortcomings such as fast clearance, low endosomal escape and insufficient nucleic acid binding (39). One of the most popular and most efficient structural modification strategies applied on polysaccharides is their conjugation with polyethylene glycol (PEG). PEG is a nonionic hydrophilic polyether and has been widely used to protect enzymatic degradation and premature clearance during circulation. The chemical modifications although have advantages including in enhancing delivery of agents by NPs, they nevertheless should not interfere in biocompatibility of NPs and, in fact, should be biologically

inert. For example, some reports assert that PEGylated NPs contrary to the impression may cause stimulating the immune system in which an intravenous injection of PEG-conjugates leads to a second dose. The immunogenicity of PEG can be due to its repeating –O–CH2–CH2– subunit (40, 41). Therefore, this limitation may take into account in the current focus on biocompatible NPs.

NPs fabricated with polysaccharides due to diversity in polysaccharides structure can overcome various existing extra- and intracellular barriers specifically in nucleic acid delivery (37). In addition, the influence of the chemical microenvironment on their physicochemical identity by interaction with macromolecules such as nucleic acids has a role in their preparation as conjugates or complexes. The mechanism of the polysaccharides-DNA complex formation involves the electrostatic interactions between cationic polymers and anionic DNA to form polyplexes. This type of complex formation provides enhanced efficacy of genes delivery to targeted cells. Furthermore, some polysaccharides owing to their intrinsic ability, are able to recognize specific cell types and hence facilitate the design of targeted delivery systems through receptor-mediated endocytosis (38).

3.1 Chitosan

Chitosan is a (1→4) 2-amino-2-deoxy-β-D-glucan that is obtained by partial alkaline deacetylation of chitin (a polysaccharide found in the exoskeleton of crustaceans and insects and the cell wall of fungi). It is a linear and cationic polysaccharide composed of randomly repeating units of D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) by β-1→4 linkages. In addition to previously mentioned ideal characteristicsof polysaccharides, because of its extensive positive charge and globular shape, chitosan is an excellent and attractive candidate among natural polysaccharides for the delivery of negatively charged nucleic

acids. It can effectively bind DNA and protect it from enzymatic degradation (42-44). Likewise, the included nucleic acid benefits from the mucoadhesive property of chitosan that permits a sustained interaction with the membrane epithelia, promoting more efficient uptake. Finally, chitosan possesses the ability to open intercellular tight junctions and thus facilitates the transport of its payload into the cells more effectively (45, 46).

Special chitosan formulations can render a promising combination therapy. These specific formulations were capable of delivering plasmid DNA and siRNA effectively to HepG2 and Caco-2 cancer cell lines leading to high levels of both GLP-1 expression and DPP-VI silencing in vitro in type 2 diabetes therapy(47). The application of an *in situ* chitosan hydrogel delivery system for osteosarcoma gene therapy combined with chemotherapy with a combination of chitosan-doxorubicin and chitosan-pigment epithelium derived factor resulted in a potential treatment for osteosarcoma and strong inhibition of tumor growth, bone lysis, and metastasis to lungs (48).

Polyionic hydrogels formed as a result of interaction between biodegradable cationic and anionic biopolymers due to the improvement of both shelf-life and half-life in biological fluids have proven remarkable characteristics for drug encapsulation and delivery. Chitosan and alginate are two biodegradable polysaccharide biopolymers that are of much interest and have been largely investigated for such applications. Alginate as a poly anionic copolymer is not applied as much as cationic polymers in nucleic acid delivery, but it improves the gene transfer by polycations. Alginate-chitosan complex is formed through interactions between the carboxyl groups of alginate and the amine groups of chitosan. This complex is stronger at lower pH values in which chitosan dissolves (49-51). Yang *et al.*, have employed chitosan-alginate nanoparticles as carriers of the pAcGFP1-C1 plasmid with an ultrasound regimen. This complex could protect the

transgene from DNase I degradation and incorporate plasmid DNA up to 600-650 nm in size with a loading efficiency of greater than 90% (52).Alginate-chitosan complex also by forming 3D scaffolds and macrocapsules facilitate gene-to-cell transfer(53). The combination of polyethylenimine (PEI) with the chitosan/DNA complex exhibited 1000 folds enhancement in gene expression in HeLa cells over that of induced by chitosan alone. Furthermore, cytotoxicity of PEI considerably decreased upon combination with the chitosan/DNA complex (54). In another study, Ho*et al.*, conjugated arginine (Arg) residues to chitosan (CS)backbone via extending arms consisting of disulfide spacers and introduced a novel non-viral carrier(CS–SS–Arg) for gene delivery in human embryonic kidney 293 (HEK 293) cell line. In this fabrication, Arg residues could efficiently condense DNA through electrostatic interactions and form CS–SS–Arg/DNA NPs with a diameter and zeta potential of 130 nm and 35 mV, respectively. Cleavage of disulfide spacers in reductive environment of cytoplasm and biodegradable properties of CS–SS–Arg led to enhanced release of DNA into the cytoplasm(55).

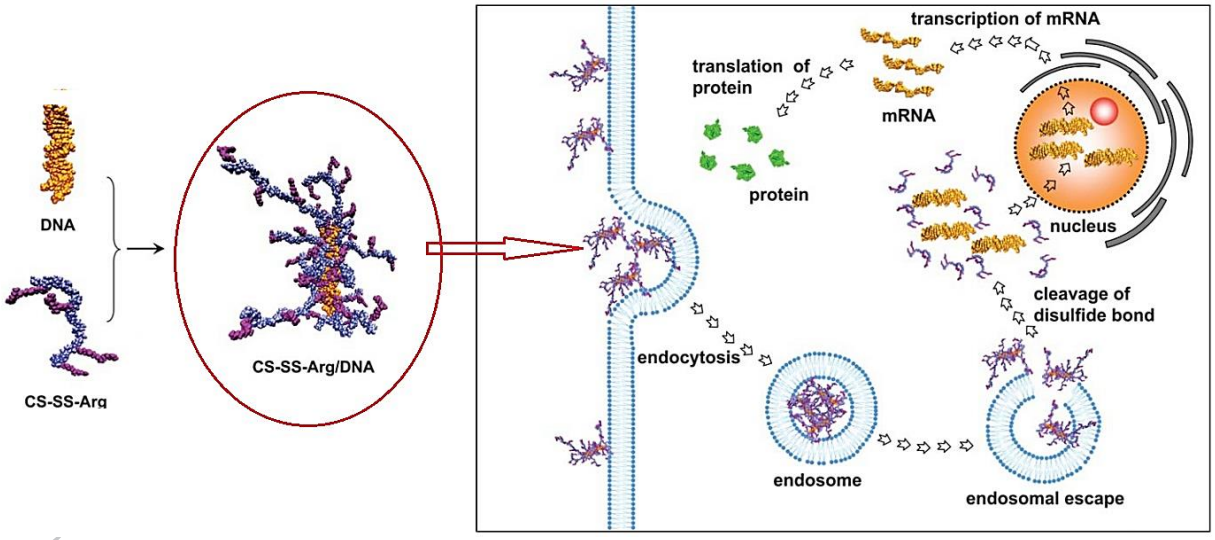

Figure 3.Schematic illustrations showing the potential mechanisms of intracellular gene expression of CS–SS–Arg/DNA nanoparticles. This figure was obtained with permission from reference(55).

3.2 Hyaluronic acid (HA)

Hyaluronic acid is a glycosaminoglycan (GAG) disaccharide occurring as a natural hydrophilic polyanionic linear polymer composed of glucuronic acid and N-acetylglucosamine repeats via a β-1→4 linkage. It exists naturally in the body with a half-life of nearly three days and comprises50% of the total resides in skin tissue (56). It can be stated that the most versatile existing macromolecule in the connective tissues of vertebrates is HA. Moreover, it is an essential and abundant constituent in some other tissues such as vitreous humor of the eye and synovial joint fluid (57).

HA is involved in different cell functions such as cell adhesion, morphogenesis, inflammation regulation, cell signaling and even transcription. It has gained a lot of interest in a great number of clinical applications. The US FDA approved it for injection for treating some diseases (58). This biopolymer, due to some advantageous and well-known features which improve transfection efficacy, has become an attractive polymer in the field of pharmaceutical technology and drug and gene delivery in recent years. Several studies have shown that establishment of complexes of polycations such as polyethylenimine (PEI) with HA, as a coating agent, could enhance gene transfection efficiency and improve their stability (59-62). For example, He *et al.* used HA as a natural anionic polysaccharide for shielding of PEI positive charge for targeted gene delivery into HepG2 and B16F10 cells expressing HA receptor. They used reducible shielding of hyaluronic acid (HA-SS-COOH) and add them to DNA/PEI complexes *via* electrostatic interaction to form ternary complexes. The shielding of DNA/PEI polyplexes with HA led to enhanced stability of NP and prevented aggregation mediated by salt and serum albumin as well as enhancement of cellular uptake by HA receptor-mediated endocytosis.

Furthermore, in the reductive environment of cytoplasm, deshielding of HA-SS-COOH led to DNA release and thereby increased the gene transfection efficiency(60).

In addition to acting as the protective coating, HA could also work as a ligand for cell targeting(63-65). Since HA is a ligand for receptors such as CD44, RHAMM, LYVE-1, and toll-like receptor 4 (TLR-4), it can be ideally employed as targeting moiety in gene carriers(37). Has with molecular weight profiles in the range of 10^3–10^7 Da have been studied in numerous applications. They possessed various physiological characteristics in different molecular weights. It has been known that HAs with low molecular weights are able to bind various cellular receptors such as CD44 which is overexpressed in normal human epithelium cells, chondrocytes and cancerous cells (66). Martens *et al*., have used HA as an electrostatic coating for polymeric gene nanomedicines, complexes of anionic plasmid DNA and the cationic N,N′-cystaminebisacrylamide-4-aminobutanol (p(CBA-ABOL) vector), for intravitreal delivery of therapeutic nucleic acids towards the retina with help of an optimized *ex vivo* model based on excised bovine eyes. Due to the presence of HA throughout the retina and as a major constituent in vitreous humor as well as presentation of CD44receptors on many retinal cell types, it might be an interesting molecule in ocular delivery for patients suffering from blinding disorders (67).

Despite all prominent features, the shortcomings of HA include both high water solubility and rapid degradation. However, its capability of interacting with positively charged materials and polymers, could enhance the stability of HA upon dilution and in the presence of serum proteins (68). For some of the target cells, HA PEGylation could lead to prolonged blood circulation time, inhibiting enzymatic degradation, improving tumor accumulation, reducing aggregation and promoting stability of the therapeutic (69).

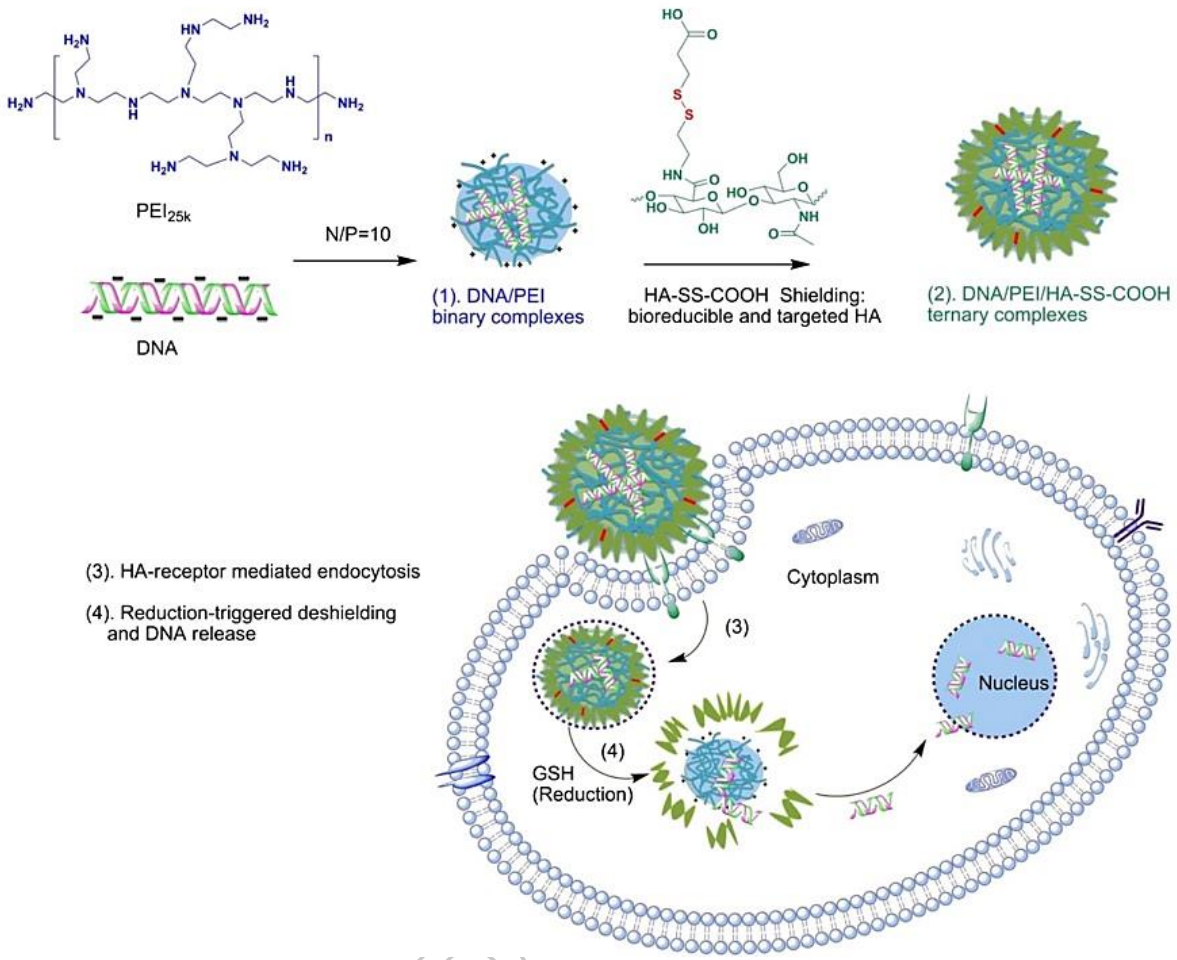

Figure 4. Schematic representation of DPS complexes for gene delivery. (1) Condensation of DNA by PEI to form DNA/PEI binary complexes (2) Shielding of DNA/PEI with bioreducible and targeted HA-SS-COOH by electrostatic interaction to construct DNA/PEI/HA-SS-COOH ternary complexes, (3) HA-receptor-mediated endocytosis and (4) reduction-triggered deshielding of HA-SS-COOH and DNA release in reductive conditions. This figure was obtained with permission from reference(60).

3.3 Dextran

Dextran is a hydrophilic nontoxic biomaterial consisting of α-1,6 linked glucopyranose units with a few 1,3-glycosidic branch linkages, which is naturally produced by some bacterial species. It is readily soluble in water as well as electrolyte solutions and it is stable for more than 5 years. Binding of dextran to erythrocytes and platelets increases their electronegativity and thus reducing erythrocytes aggregation. Dextran has been used as a plasma volume expander and a

blood flow adjuvant (70). It is also applied to stabilize enzymes. Owing to several attractive properties and high safety, it can be used for both parenteral and oral administrations. This is confirmed by its continuous use in clinics and pharmacy (71). In the field of gene therapy, many attempts have been made to investigate the dextran and its derivatives. Native dextrans, charged dextrans(dextran sulfate or diethylaminoethyl (DEAE)-dextran) and hydrophobically-modified dextran have been studied for pharmaceutical applications to coat some polymeric particles and liposomal vesicles. Dextran sulfate is a polymer that has been widely used for preparing nanosized polyelectrolyte complexes in different fields(37). Additionally, dextran is employed desirably as a carrier for encapsulation of nucleic acids. Raemdonck*et al.*, successfully designed biodegradable cationic dextran nanogels for controlled-release delivery of siRNA in HuH-7 human hepatoma cells by enhancing the endosomal escape mechanism and tailoring the degradation kinetics of nanogels inside the cells. These nanoparticles could encapsulate siRNA by electrostatic interactions(72). It has been found that the incorporation of polyanions such as dextran sulfate or hyaluronic acid resulted in more compact siRNA polyplexes compared to complexes with polycations alone (73).

Different modifications of dextran structure have overcome some of its inherent drawbacks in delivery systems. One of the major drawbacks of dextran is its high polarity which may exclude its trans cellular passage. Another problem is its susceptibility to enzymatic degradation in the human body (74). Conjugations of dextran with moieties such as PEI, glycidyl trimethyl ammonium chloride (GTAC), diethylaminoethyl (DEAE), spermine and protamine were found to be of great importance in increased cytotoxicity and enhanced transfection efficiency (75). Linear dextran bearing a high amount of hydroxyl side groups is amenable to efficient chemical modifications. For example, partial oxidation of dextran hydroxyl groups to form aldehydes

would enable grafting of branched PEI. Thomas and co-workers designed a cationic dextran derivative using protamine in order to evaluate target specific cellular binding. Disuccinidyl carbamate and 4-methylaminopyridine were used for activation of the hydroxyl groups followed byconjugation to the amino group of protamine. The transfection efficiency of the polymer/DNA complex represented capability of modified dextran in high gene expression and cellular uptake in HepG2 cells (76).

3.4 **Other polysaccharides**

There are lesserknown polysaccharides, which can also be applied in gene therapy. Among these, polymers such as alginate, cyclodextrins, β-glucans, arabinogalactan, pullulan and pectin could be mentioned. Alginate is more applicable in drug delivery systems. It is an unbranched polyanionic copolymer, consists of (1-4) linked β-D-mannuronate and α-L-guluronate residues in homopolymer or heteropolymer block structures. It is widely used as biomaterials, especially in tissue engineering and regeneration and as a carrier for controlled release systems. It has also chelating capabilities. The US FDA has approved alginates for use as polymers. Nevertheless, there are few reports about its usage as nucleic acids carrier. Similar to hyaluronic acid and dextran, alginate has also been exploited in alleviating PEI-mediated cytotoxicity (37, 38). Jiang *et al.* indicated that PEI/DNA polyplexes with an alginate coating enhanced reporter gene expression *in vivo* in comparison to the uncoated complexes. The results of this study demonstrated that the anionic alginate coating of the DNA/PEI polyplexes contributed to efficient gene delivery *in vitro* and *in vivo*(77).

4 Polyesters

Polyesters are produced by polymerizing a polyhydric alcohol along with the addition of apolybasic acid that could bear hydrolysable backbone (78). Properties of these polymers depend on some factors such as monomer composition, mean molecular weight, polydispersity and glass

transition temperature (79). Biodegradable polyesters can be classified into two main groups; polymers either derived from microorganisms or synthetically made from natural or synthetic monomers (80). The biodegradable polyesters in a different classification include poly (lactic acid), polyhydroxyalkanoates with agro-resources, polycaprolactone, biodegradable aliphatic polyesters, aromatic copolyesters and polyesteramide with petroleum resources (81).

Polyesters have been widely explored in biomedical applications such as tissue engineering, controlled drug delivery of both hydrophobic and hydrophilic drugs, sutures and implants (82). Notwithstanding that polyesters have excellent tissue compatibility; their hydrophobicity can be a barrier in medical applications including their use as gene delivery agents. Hence, polyesters conjugated with other cationic polymers, or chemically modified in structure will gain the capability of forming polyionic complexes through electrostatic interactions with DNA. Furthermore, functionalizing of aliphatic polyesters imparts other useful properties to modified complex(83, 84). On the contrary to some counterpart vectors employed for gene delivery, polyesters have several advantages including low immunogenicity and toxicity, high DNA-carrying capacity, the simplicity of large-scale production and long-term delivery of therapeutic agents. Moreover, their degradation into low molecular weight products would facilitate their renal clearance (85-87).

Nevertheless, for increasing the efficiency of gene delivery, reducing cytotoxicity and dependent degradation in addition to passive and/or active targeting, different derivatives of biodegradable polyesters have to be produced, which is further discussed in the following sections (88).

4.1 Polylactic acid or polylactide (PLA)

PLA is a hydrophobic polyester chiral molecule that is present as two stereoisomers; L- and D-lactic acid, which can be produced in two ways. One method involves biological production

through lactic bacteria mainly related to the genus lactobacillus or fungi and the second approach is chemical production using renewable sources (81). Inherent properties of PLAs such as biocompatibility, biodegradability, mechanical strength, heat processability, solubility in organic solvents, microparticles (MP) and nanoparticles (NP) of PLA have been progressively employed as systems for different macromolecules delivery(89). However, many researchers introduce dependent groups in the polymer backbone for improving the efficiency of delivery systems because the majority of PLA polymers suffer from lack of any functional groups in their backbone. Li and Huang synthesized copolymer namely poly[(D,L-lactide-co-4-hydroxy-L-proline) (PLPH)] using ring-opening polymerization of D,L-lactide with N-cbz-4-hydroxy-L-proline in the presence of stannous octoate. They revealed that PLHP/pDNA was able to perform multiple functions such as controlled degradation rate, negligible cytotoxicity along with containing a functional group for further conjugation with targeting ligands (90).

Liu *et al.*, attached folate (Fa) to poly(ethylene glycol)-b-poly(D, L-lactide) (PEG-PLA) to form Fa-PEG-PLA conjugate which binds to receptors on the cell surfaces in order to increase the cellular uptake. *In vitro* transfection efficiency of Fa-NPs/DNA was evaluated in HeLa cells and human umbilical vein endothelial cells. Their findings showed that Fa-PEG-PLA NPs could function as an excellent carrier for gene loading and delivery and could be considered as tumor cell-targeted medicine for the treatment of cervical cancer (91).

Another approach for improving the properties of PLA as gene carrier is to conjugate it with an amphiphilic and cationic lipid. Yang *et al.* showed that cationic lipid-assisted poly(ethylene glycol)-b-poly(D,L-lactide) (PEG–PLA) nanoparticles containing siRNA against the Polo-Like kinase-1 (PLK1) gene could induce remarkable apoptosis in both HepG2 and MDA-MB-435s

cancer cells. On the other hand, systemic delivery of PEG–PLA nanoparticles loaded with the same siRNA suppressed tumor growth in a MDA-MB-435 murine xenograft model (92).

In another study, siRNAs against Aldh1a2 (retinoic acid (RA)-synthesizing enzyme) and dusp6 (also known as MAP-kinase phosphatase, mkp3) were encapsulated in cationic lipids (BHEM-Chol) and PEG–PLA.The siRNA-encapsulated nanoparticles successfully entered the cells and resulted in are markable gene-specific knockdown in adult zebrafish heart (as an important model organism for studying heart regeneration)(93).

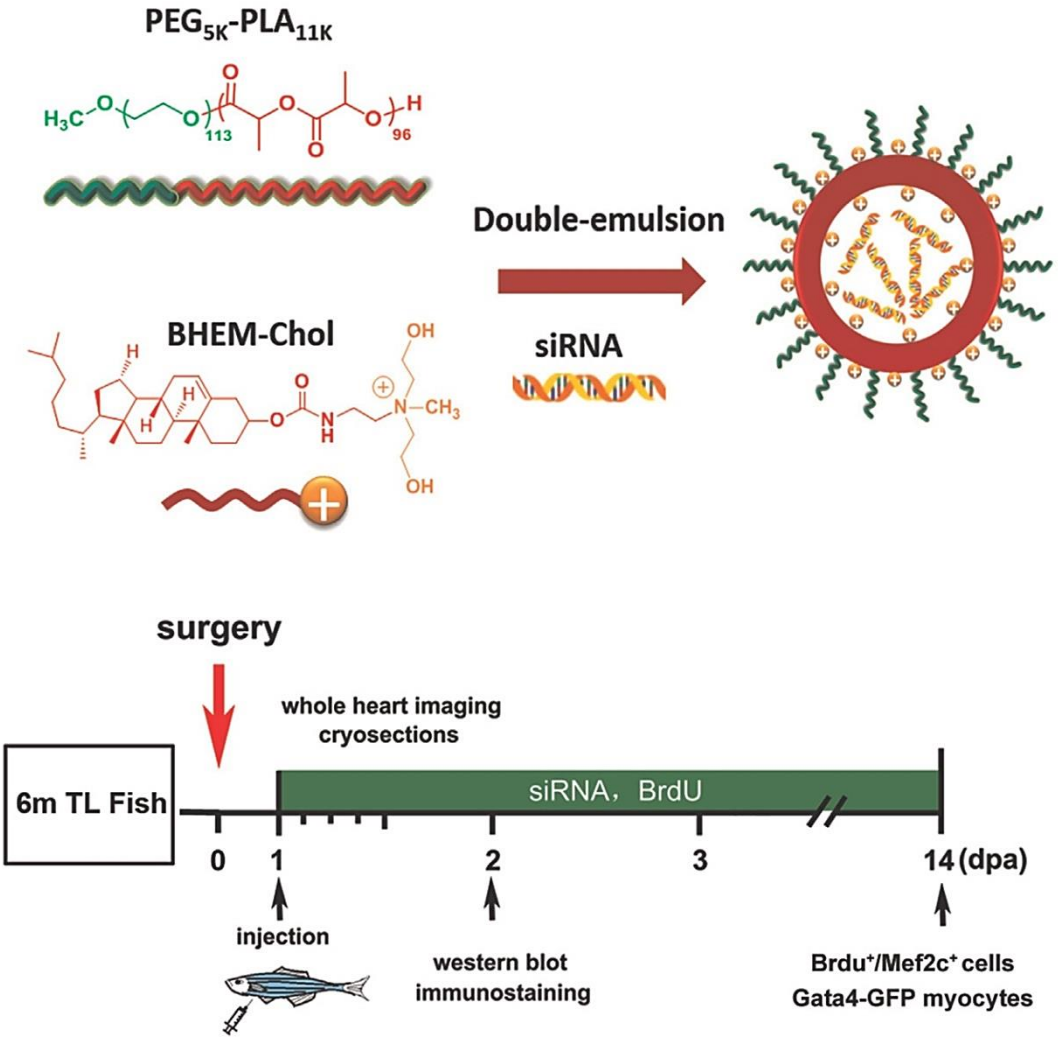

Figure 5. Overview of preparation and delivery of nanoparticle-encapsulated siRNAs into zebra fish hearts after ventricular resection. This figure was obtained with permission from reference(93).

4.2 Poly (lactide-co-glycolide) (PLGA)

PLGA is aliphatic biodegradable copolyester which is synthesized by means of ring-opening co-polymerization of two different monomers, namely lactic and glycolic acids. The combination of polylactic acid (PLA) and polyglycolic acid (PGA) yields one of the most successfully developed biocompatible and biodegradable polymers, especially in drug delivery. Depending on the ratio of lactate to glycolate used for the polymerization, different forms of PLGA can be produced (78, 94).

PLGA is a biodegradable and biocompatible copolymer which was approved by the European Medicine Agency (EMA) and the US Food and Drug Administration (FDA) for implants, parenteral microspheres and periodontal drug-delivery(95-97). The hydrolysis of their ester linkages and subsequent biodegradation of PLGA in body releases two non-toxic metabolite monomers, lactic and glycolic acids which easily metabolized through the Krebs cycle, resulting in minimal systemic toxicity(98, 99). The rate of degradation is related to several factors including the monomer ratio of PLGA, the degree of crystallinity, molecular weight, and the glass transition temperature (Tg) of the copolymer(100, 101). For example, the polymer composed of 50% lactic acid and 50% glycolic acid is hydrolyzed much faster than those containing an unequal ratio of monomers(100, 102).

PLGA copolymers have useful properties such as decomposition to nontoxic by-products, mechanical resistance, and regular individual chain geometry, as well as controlled rate of degradation (103). Although PLGA nanoparticles could protect the encapsulated DNA from *in vivo* degradation but there are many challenges involved in the application of PLGA as gene

carriers such as poor encapsulation efficiency, insufficient lysosomal escape, and low cellular uptake.

Many studies have been done to improve the properties of PLGA nanoparticles as gene delivery vector by conjugating it with other polymers such as polyethyleneglycol(PEG), polyethylenimine(104), poly-L-lysine(105), polyamidoamine and chitosan(106).These polymers could enhance cellular uptake, buffering capacity and endosomal escape of PLGA NPs. PEGylation of PLGA NPs also increases their solubility and stability, blood circulation half-life, decreases immunogenicity, reduces intermolecular aggregation and finally avoids recognition of NPs by reticulo-endothelial system (RES) (96, 97, 107).

Targeting of PLGA NPs is another approach to efficient and specific delivery of therapeutic genes to a target site. Cationic PLGA was modified with asialofetuin (AF) whichis known as an excellent ligand molecule selectively recognized by the asialoglycoprotein receptor (ASGPr) on hepatocyte cells. The results showed that targeted asialofetuin-PLGA conjugates carrying genes encoding for luciferase and interleukin-12 (IL-12) could increase transfection efficiency compared to free DNA and non-targeted systems in cultured HeLa cells(108).

4.3 Polycaprolactone (PCL)

PCL is a polymer existed in petroleum sources, which is normally produced by ring-opening polymerization of ε-caprolactone in the presence of metal alkoxides(109). Nanoparticles made from PCL have a promising place in biomedical applications for their high colloidal stability in a biological fluid, facile cellular uptake by endocytosis, low toxicity *in vitro* and *in vivo*, and controlled release of their cargo (110). PCL nanoparticles can be effective carriers for RNAs because of high stability and stealth properties. Palamà*et al.*, in a study conducted on the effect

of mRNA-protamine complex encapsulated PCL nanoparticles in the intracellular delivery of mRNA molecules, reported that efficiency of mRNA on transfected NIH 3T3 fibroblasts, HeLa cells, and MG63 osteoblasts led to higher loading efficiency, better stability, and controlled release of the mRNA over time (111). Diao *et al.* improved oligodendroglial precursor cell(OPC) differentiation and maturation using miR-219 and miR-338 incorporated into PCL nanofibers(112).

4.4 Polyhydroxyalkanoates (PHAs)

PHAs are a family of biodegradable, non-toxic, biocompatible and natural linear polyesters, which are synthesized using a wide variety of bacteria (113). The synthesis of biodegradable cationic copolymers containing PHAs such as Poly [(R)-3-hydroxybutyrate] (PHB), produces gene delivery vectors acquiring good water solubility. Polyethylenimine (PEI) and poly(2-dimethylamino)ethyl methacrylate) (PDMAEMA) as a cationic polymer can be used to design such copolymers. PDMAEMA-PHB-PDMAEMA triblock copolymers have been demostrated that bear significantly lower toxicity and more efficient gene transfection as compared to PEI and PDMAEMA homopolymers. This copolymer had strong condensation ability for negatively charged plasmid DNA (pDNA) to form copolymer/pDNA polyplexes which resulted in an excellent gene transfection in COS-7 and HEK293 cells(114). Accordingly, for PHAs to be translated into therapeutic applications, they need to be chemicallly functionalized especially for imparting hydrophilic property(115). A (PHB-b-PEG-NH2) nanoparticle platform was prepared via trans-esterification reactions to be able to condense nucleic acids and to be applicable as a delivery system. This carrier system was synthesized for protection from enzymatic degradation, increased intracellular capture and improved delivery(116).

5 Polyamides

Polyamides are biopolymers with repeating units held together by amide bonds. They either occur naturally or artificially. The well-known and typical examples are protein and nylon, respectively. Polyamides have different industrial and biomedical applications which include water softener, feed preservative, cosmetics, and bio molecular delivery (117). Usually, polyamides applied in controlled delivery systems are synthesized in microorganisms by enzymatic processes independently from ribosomal protein biosynthesis. These non-ribosomally synthesized biopolymers occur in several bacteria mainly in*Bacillus* spp. These are referred to as poly(amino acids) in order to distinguish them from the proteins (118). They have been employed in the delivery of therapeutic molecules because they possess properties such as biocompatibility, biodegradability, and non-toxicity. In comparison to other potent vectors, families of polyamides such as cationic poly(amido amine)s have been studied considerably as polymeric gene delivery vectors. Upon binding to nucleic acids, these polymers can form nanosizedpolyplexes and mediate their endosomal escape and thereby inducing improved transfection efficiency (119).

5.1 Poly(ε-L-lysine) (PLL)

PLL with a biodegradable polymer backbone in which the α-carboxyl group is linked to the ε-amino group of lysine is a cationic polymer at neutral pH and is widely used as an antimicrobial agent for preservation of animal feed (118). Upon formation a complex of nucleic acid-poly-lysine-ligand (polyplex), the genetic material transfer is facilitated *via* receptor-mediated endocytosis. It is known that PLLs have a poor endosomal escape (pH 5–6.5) and thus there is a probable subsequent degradation of the nucleic acid cargo in the late lysosomes (pH ~4.5).

However, PLL has protonated amino groups with high charge density, controllable molecular size, and shape as well as a potential flexibility for chemical modifications, and thereby can be considered as a promising candidate for gene delivery. To assist endosomal escape, various modifications of PLL such as attachment of fusogenic/synthetic peptides and pH-sensitive moieties can be helpful (120). Gue*et al.*, by using of a pH-triggered amphiphilic poly-L-lysine nanocarrier delivered therapeutic small interfering RNA to suppress prostate cancer growth in mice. Modification of polymers with PEG chains can prevent their non-specific binding to serum proteins and particle self-aggregation which results in longer blood circulation times. In this work, PEGylation of poly-L-lysine modified with cholic acid (PLL-CA), was investigated for siRNA delivery. These nanoparticles have the capability of entering the inflammation sites as well as solid tumors and attaining to reduced liver filtration. PEGylation of these amphiphilic PLL nanoparticles improved the delivery potential of the materials in the various ways (120). In addition, PLL has excellent plasmid DNA (pDNA) condensation capacity. To overcome the relatively high cytotoxicity and low transfection efficiency of PLL, the group of Zhoua synthesized well-defined glycopolymers by reversible addition-fragmentation transfer polymerization which was then grafted onto PLL. These modified polymers can be used to condense pDNA with proper strength, which can protect DNA from enzyme degradation and consequent release of the condensed pDNA inside the cells. Transfection of NIH3T3 and HepG2 cells showed improved transfection efficiencies (121).

5.2 Poly(γ-glutamate) (PGA)

PGA is a water-soluble, anionic, biodegradable non-toxic polyamide which consists of glutamic acid repeats with amide linkages between the α-amino and γ-carboxyl groups. It is a material

widely used in industry as thickener, cryoprotectant, sustained release material, drug carrier, curable biological adhesive, highly water-absorbable hydrogels and heavy metal absorbers (122). Although polycationic vectors can easily interact with DNA molecules and condense them effectively to form polyelectrolyte complexes (polyplex), but binding non-specifically to negatively charged proteoglycans available on cell membranes and aggregation with blood components due to the presence of strong cationic surface charges, can be major barriers to using them as controlled delivery carriers. Accordingly, several strategies such as covering the cationic polymers surface by polymers such as PEG and polyvinylpyrrolidine or reducing the surface charges and recharging them with anionic compounds such as PGA have been successfully led to enhanced transfection efficiency (123). In one *in vivo* study, liposome/siRNA complexes (lipoplexes) coated with chondroitin sulfate C, poly-L-glutamic acid and poly-aspartic acid for siRNA delivery by intravenous injection were developed to evaluate the biodistribution and gene silencing effect in mice. The findings showed that PGA coatings for cationic lipoplex containing cholesterol-modified apolipoprotein B siRNA might induce accumulation in the liver and suppress the liver-specific apolipoprotein B mRNA level(123). Peng*et al.*, in an in vitro study on HT1080 (human fibrosarcoma) cells evaluated cellular uptake and transfection efficiency of poly(γ-glutamic acid) chitosan/DNA nanoparticles. After incorporating γ-PGA in chitosan/DNA complexes, a significant increase in transfection efficiency was observed. Significantly enhanced cellular uptake, the presence of specific trypsin-cleavable proteins involved in the internalization of these complex nanoparticles as well as improving the release of DNA intracellularly were observed(124).

6 **Polyanhydrides**

Polyanhydrides are a class of biodegradable polymeric carriers, which specified by repeat units of the polymer backbone chain linked by anhydride bonds. The instability of the anhydride bond allows the degradation of polyanhydrides into non-toxic diacid monomers and hence they are considered biocompatible. They have been considered extensively as useful biomaterials in drug delivery to various organs of the human body such as the brain, bone, blood vessels, and eyes (125, 126). Inorganic polyphosphates are linear polymers formed by linking orthophosphate units (PO_4) by high-energy phosphoanhydride bonds. They are the only polyanhydrides present in all living cells from bacteria to mammals (127). Polyphosphates have received particular attention in biomedicine. Excellent biocompatibility and biodegradability, similarity to biomacromolecules such as nucleic acids, as well as the presence of pentavalent phosphorus that possesses potent covalent linking to the drugs, offers them as fascinating drug delivery systems. They also by the convenient functionalization of phosphorus can indicate good flexibility (128-130). In spite of the fact that very few studies have been reported concerning the utility of polyphosphates in the area of gene therapy, however, functionalization of polyphosphates is a popular topic to improve the performance of the controlled release of genes. In one study, evaluation of L-tyrosine polyphosphate-plasmid DNA (LTP-pDNA) nanoparticles in an *in vivo* setting was implemented *via* injection into rodent uterine tissue. In this study, Ditto *et al.* showed that nanoparticles formulated from an amino acid based polyphosphate polymer encoding for theβ-gal gene in *E. coli* have successfully transfected the uterus in an *in vivo* rat model (131). Furthermore, polyphosphates have been explored to reduce the cytotoxicity of other nonviral vectors such as polyethylenimine by masking of the high cationic surface charge of PEI. Huang *et.al.,* showed that polyethylenimine–tripolyphosphate nanoparticles have significant transfection efficiency than polyethylenimineal one for both pDNA and siRNA delivery in different cell lines

while conferring little or no toxicity in the cells, and thus polyphosphates could improve the performance of PEI as gene delivery vector(132).

7. Conclusion

Polymers with biodegradability property such as polymers consisting of carbohydrate, ester and amide backbones produce natural byproducts such as water and organic acids after being broken down. On the other hand, biocompatible polymers produce desirable effects without causing unwanted host responses making them suitable as gene or drug carriers. That is why biodegradable and biocompatible polymers have raised great interest in the biomedical fields. More interestingly, these polymers are inert towards their cargos owing to their proper size and charge density which offer them as ideal delivery vehicles in the recent years. They have been able to significantly solve the delivery challenges in nucleic acids-based therapy as an excellent approach for the treatment of many genetic diseases. MOREOVER, as multifunctional nano-scale carriers, they have shown promising results in gene delivery applications. It is expected that by applying various effective strategies including improving functional quality of the biodegradable polymers, the future direction will be translation of these polymers as carriers for gene and drug delivery applications.

8. Expert opinion

There is a growing interest in developing chemically based nanomaterials for oligonucleotide delivery. However, most of these materials are not as efficient as viral vectors in gene transfer activity and are neither safe nor their fate in cells/tissues clearly known. Biocompatible and

biodegradable polymers represent excellent choices addressing the safety problems usually encountered with either viral or other non-viral vectors in oligonucleotide delivery. Despite recent improvements to the gene transfer ability of biocompatible and biodegradable polymers but the biggest challenge ahead is to greatly improve on the gene transfection activity of these carriers so that they can be translated into clinical applications.

The most important issue that still requires special attention and would determine the future direction of research in this filed constitutes structural modifications of currently available biocompatible and biodegradables. There are numerous biodegradable and biocompatible polymers with different physicochemical characters and we believe that a single material will not satisfy the entire design criteria, thus one approach would be to employ advantages of each biocompatible polymer and build these advantages into one hybrid structure by which it would be expected to improve on the transfection efficiency of the gene carrier without compromising the safety issues. Current trend in polymeric gene carriers is the interest in multifunctional nano-scale carriers. The basic principles for gene carriers to be translated into clinical applications include not only exhibit efficient gene transfer activity, but in addition, it could have a hydrophilic corona for prolonged circulation time after being injected although it may deteriorate the gene transfer activity of the carrier as has been observed with PEGylation. It may also be decorated with a ligand such as peptides, antibodies, or aptamers to deliver the oligonucleotide payloads to the target cells which could potentially enhance the transfection efficiency of the carriers and reduce the possible side effects. Although there are many reports on the targeted gene delivery into cells using biocompatible and biodegradable polymers, but there are many other targeting ligands that have not been tested yet. The carrier may possess a chemical entity to pass through the cell membrane in order to efficiently reach the cytosol or an intracellular target.

The polymeric carrier may as well being conjugated to a contrast agent to allow for visualization *in vivo* after injection, if necessary, and have a pH-sensitive function to control the release of the gene payload under acidic pH of endosomal compartment. Future works on biodegradable and biocompatible carriers will have to focus on optimizing not only the gene transfer activity but also to modify their structures such that they efficiently condense DNA, are able to better enter the target cells, are released efficiently from endosomal compartment into the cytosol, traffic through cytosol toward nucleus prefery and eventually enter the nucleus for being transcribed. Thus, biocompatible and biodegradable polymers will play a necessary and important role in developing new and safe carriers for oligonucleotide delivery.

Expert Opinion on Biological Therapy

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

References:

1. Niidome T, Huang L. Gene therapy progress and prospects: nonviral vectors. Gene therapy. 2002;9(24):1647-52.
●● Highly informative that provides an exhaustive account about gene therapy.
2. Mokhtarzadeh A, Parhiz H, Hashemi M, Ayatollahi S, Abnous K, Ramezani M. Targeted Gene Delivery to MCF-7 Cells Using Peptide-Conjugated Polyethylenimine. AAPS PharmSciTech. 2015:1-8.
3. Verma IM, Somia N. Gene therapy-promises, problems and prospects. Nature. 1997;389(6648):239-42.

4. Luten J, van Nostrum CF, De Smedt SC, Hennink WE. Biodegradable polymers as non-viral carriers for plasmid DNA delivery. Journal of Controlled Release. 2008;126(2):97-110.
5. Friedmann T, Roblin R. Gene therapy for human genetic disease? Science. 1972;175(4025):949-55.
6. Davis ME. Non-viral gene delivery systems. Current opinion in biotechnology. 2002;13(2):128-31.
●●An overview article provides an exhaustive account on nonviral vectors for gene delivery
7. Ginn SL, Alexander IE, Edelstein ML, Abedi MR, Wixon J. Gene therapy clinical trials worldwide to 2012–an update. The journal of gene medicine. 2013;15(2):65-77.
8. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. Nature Reviews Genetics. 2014;15(8):541-55.
●●An overview article provides an exhaustive account on nonviral vectors for gene delivery.
9. Hashemi M, Parhiz H, Mokhtarzadeh A, Tabatabai SM, Farzad SA, Shirvan HR, et al. Preparation of Effective and Safe Gene Carriers by Grafting Alkyl Chains to Generation 5 Polypropyleneimine. AAPS PharmSciTech. 2015:1-11.
10. Wang W, Li W, Ma N, Steinhoff G. Non-viral gene delivery methods. Current pharmaceutical biotechnology. 2013;14(1):46-60.
11. Hashemi M, Ayatollahi S, Parhiz H, Mokhtarzadeh A, Javidi S, Ramezani M. PEGylation of Polypropylenimine Dendrimer with Alkylcarboxylate Chain Linkage to Improve DNA Delivery and Cytotoxicity. Applied biochemistry and biotechnology. 2015;177(1):1-17.
12. Albanese A, Tang PS, Chan WC. The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annual review of biomedical engineering. 2012;14:1-16.
13. Papasani MR, Wang G, Hill RA. Gold nanoparticles: the importance of physiological principles to devise strategies for targeted drug delivery. Nanomedicine: Nanotechnology, Biology and Medicine. 2012;8(6):804-14.
14. Khansarizadeh M, Mokhtarzadeh A, Rashedinia M, Taghdisi S, Lari P, Abnous K, et al. Identification of possible cytotoxicity mechanism of polyethylenimine by proteomics analysis. Human & experimental toxicology. 2015:0960327115591371.
15. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Advanced drug delivery reviews. 2003;55(3):329-47.
16. Behnam B, Shier WT, Nia AH, Abnous K, Ramezani M. Non-covalent functionalization of single-walled carbon nanotubes with modified polyethyleneimines for efficient gene delivery. International journal of pharmaceutics. 2013;454(1):204-15.
17. Sun X, Liu Z, Welsher K, Robinson JT, Goodwin A, Zaric S, et al. Nano-graphene oxide for cellular imaging and drug delivery. Nano research. 2008;1(3):203-12.
18. Ayatollahi S, Hashemi M, Oskuee RK, Salmasi Z, Mokhtarzadeh A, Alibolandi M, et al. Synthesis of efficient gene delivery systems by grafting pegylated alkylcarboxylate chains to PAMAM dendrimers: Evaluation of transfection efficiency and cytotoxicity in cancerous and mesenchymal stem cells. Journal of biomaterials applications. 2015:0885328215599667.

19. Ramezani M, Khoshhamdam M, Dehshahri A, Malaekeh-Nikouei B. The influence of size, lipid composition and bilayer fluidity of cationic liposomes on the transfection efficiency of nanolipoplexes. Colloids and Surfaces B: Biointerfaces. 2009;72(1):1-5.
20. Chen AA, Derfus AM, Khetani SR, Bhatia SN. Quantum dots to monitor RNAi delivery and improve gene silencing. Nucleic acids research. 2005;33(22):e190-e.
21. Danesh NM, Lavaee P, Ramezani M, Abnous K, Taghdisi SM. Targeted and controlled release delivery of daunorubicin to T-cell acute lymphoblastic leukemia by aptamer-modified gold nanoparticles. International journal of pharmaceutics. 2015;489(1):311-7.
22. Al-Dosari MS, Gao X. Nonviral gene delivery: principle, limitations, and recent progress. The AAPS journal. 2009;11(4):671-81.
23. Cuenca AG, Jiang H, Hochwald SN, Delano M, Cance WG, Grobmyer SR. Emerging implications of nanotechnology on cancer diagnostics and therapeutics. Cancer. 2006;107(3):459-66.
24. Misra R, Acharya S, Sahoo SK. Cancer nanotechnology: application of nanotechnology in cancer therapy. Drug Discovery Today. 2010;15(19):842-50.
25. Soltani F, Parhiz H, Mokhtarzadeh A, Ramezani M. Synthetic and biological vesicular nano-carriers designed for gene delivery. Current pharmaceutical design. 2015.
26. Kohane DS, Langer R. Biocompatibility and drug delivery systems. Chem Sci. 2010;1(4):441-6.
27. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. Advanced drug delivery reviews. 2002;54(5):631-51.
28. Mahapatro A, Singh DK. Biodegradable nanoparticles are excellent vehicle for site directed in-vivo delivery of drugs and vaccines. Journal of nanobiotechnology. 2011;9:55.
29. Naahidi S, Jafari M, Edalat F, Raymond K, Khademhosseini A, Chen P. Biocompatibility of engineered nanoparticles for drug delivery. Journal of controlled release. 2013;166(2):182-94.
30. Mokhtarzadeh A, Parhiz H, Hashemi M, Abnous K, Ramezani M. P53-Derived peptides conjugation to PEI: an approach to producing versatile and highly efficient targeted gene delivery carriers into cancer cells. Expert Opin Drug Deliv. 2016:1-15.
31. Kang ML, Im G-I. Drug delivery systems for intra-articular treatment of osteoarthritis. Expert opinion on drug delivery. 2014;11(2):269-82.
32. Yoshioka Y, Higashisaka K, Tsutsumi Y. Biocompatibility of Nanomaterials. Nanomaterials in Pharmacology.185-99.
33. McNeil SE. Nanoparticle therapeutics: a personal perspective. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology. 2009;1(3):264-71.
34. Gu J, Fang X, Hao J, Sha X. Reversal of P-glycoprotein-mediated multidrug resistance by CD44 antibody-targeted nanocomplexes for short hairpin RNA-encoding plasmid DNA delivery. Biomaterials. 2015;45:99-114.

35. Shahidi-Hamedani N, Shier WT, Moghadam Ariaee F, Abnous K, Ramezani M. Targeted gene delivery with noncovalent electrostatic conjugates of sgc-8c aptamer and polyethylenimine. The journal of gene medicine. 2013;15(6-7):261-9.
36. Liu Z, Jiao Y, Wang Y, Zhou C, Zhang Z. Polysaccharides-based nanoparticles as drug delivery systems. Adv Drug Deliv Rev. 2008;60(15):1650-62.
37. Raemdonck K, Martens TF, Braeckmans K, Demeester J, De Smedt SC. Polysaccharide-based nucleic acid nanoformulations. Adv Drug Deliv Rev. 2013;65(9):1123-47.
●● This is an exhaustive review that highlights various polysaccharides and their derivatives, currently employed in the design of nucleic acid nanocarriers.

38. Uthaman S, Lee SJ. Polysaccharide-Coated Magnetic Nanoparticles for Imaging and Gene Therapy. 2015;2015:959175.
39. Cumpstey I. Chemical modification of polysaccharides. ISRN Org Chem. 2013;2013:417672.
40. Wang C, Cheng X, Su Y, Pei Y, Song Y, Jiao J, et al. Accelerated blood clearance phenomenon upon cross-administration of PEGylated nanocarriers in beagle dogs. International Journal of Nanomedicine. 2015;10:3533-45.
41. Verhoef JJF, Anchordoquy TJ. Questioning the Use of PEGylation for Drug Delivery. Drug delivery and translational research. 2013;3(6):499-503.
42. Mansouri S, Lavigne P, Corsi K, Benderdour M, Beaumont E, Fernandes JC. Chitosan-DNA nanoparticles as non-viral vectors in gene therapy: strategies to improve transfection efficacy. Eur J Pharm Biopharm. 2004;57(1):1-8.
● This paper summarize the state of the art in gene therapy and particularly the use of chitosan to improve the transfection efficiency in vivo and in vitro.

43. Tong H, Qin S, Fernandes JC, Li L, Dai K, Zhang X. Progress and prospects of chitosan and its derivatives as non-viral gene vectors in gene therapy. Curr Gene Ther. 2009;9(6):495-502.
44. Köping-Höggård M, Mel'nikova YS, Vårum KM, Lindman B, Artursson P. Relationship between the physical shape and the efficiency of oligomeric chitosan as a gene delivery system in vitro and in vivo. The journal of gene medicine. 2003;5(2):130-41.
45. Illum L. Chitosan and its use as a pharmaceutical excipient. Pharm Res. 1998;15(9):1326-31.
46. Takeuchi H, Yamamoto H, Niwa T, Hino T, Kawashima Y. Enteral absorption of insulin in rats from mucoadhesive chitosan-coated liposomes. Pharm Res. 1996;13(6):896-901.
47. Jean M, Alameh M, De Jesus D, Thibault M, Lavertu M, Darras V, et al. Chitosan-based therapeutic nanoparticles for combination gene therapy and gene silencing of in vitro cell lines relevant to type 2 diabetes. Eur J Pharm Sci. 2012;45(1-2):138-49.

48. Ta HT, Dass CR, Larson I, Choong PF, Dunstan DE. A chitosan hydrogel delivery system for osteosarcoma gene therapy with pigment epithelium-derived factor combined with chemotherapy. Biomaterials. 2009;30(27):4815-23.
49. Chellat F, Tabrizian M, Dumitriu S, Chornet E, Magny P, Rivard CH, et al. In vitro and in vivo biocompatibility of chitosan-xanthan polyionic complex. J Biomed Mater Res. 2000;51(1):107-16.
50. Gazori T, Khoshayand MR, Azizi E, Yazdizade P, Nomani A, Haririan I. Evaluation of Alginate/Chitosan nanoparticles as antisense delivery vector: Formulation, optimization and in vitro characterization. Carbohydrate Polymers. 2009;77(3):599-606.
51. Hamman JH. Chitosan based polyelectrolyte complexes as potential carrier materials in drug delivery systems. Mar Drugs. 2010;8(4):1305-22.
52. Yang SJ, Chang SM, Tsai KC, Chen WS, Lin FH, Shieh MJ. Effect of chitosan-alginate nanoparticles and ultrasound on the efficiency of gene transfection of human cancer cells. J Gene Med. 2010;12(2):168-79.
53. Green DW, Kim EJ, Jung HS. Spontaneous gene transfection of human bone cells using 3D mineralized alginate-chitosan macrocapsules. J Biomed Mater Res A. 2015;103(9):2855-63.
54. Zhao QQ, Chen JL, Han M, Liang WQ, Tabata Y, Gao JQ. Combination of poly(ethylenimine) and chitosan induces high gene transfection efficiency and low cytotoxicity. J Biosci Bioeng. 2008;105(1):65-8.
55. Ho Y-C, Liao Z-X, Panda N, Tang D-W, Yu S-H, Mi F-L, et al. Self-organized nanoparticles prepared by guanidine-and disulfide-modified chitosan as a gene delivery carrier. Journal of Materials Chemistry. 2011;21(42):16918-27.
56. Pure E, Assoian RK. Rheostatic signaling by CD44 and hyaluronan. Cell Signal. 2009;21(5):651-5.
57. Calabro A, Oken MM, Hascall VC, Masellis AM. Characterization of hyaluronan synthase expression and hyaluronan synthesis in bone marrow mesenchymal progenitor cells: predominant expression of HAS1 mRNA and up-regulated hyaluronan synthesis in bone marrow cells derived from multiple myeloma patients. Blood. 2002;100(7):2578-85.
58. Ascher B, Cerceau M, Baspeyras M, Rossi B. [Soft tissue filling with hyaluronic acid]. Ann Chir Plast Esthet. 2004;49(5):465-85.
59. Chen JX, Wang M, Tian HH, Chen JH. Hyaluronic acid and polyethylenimine self-assembled polyion complexes as pH-sensitive drug carrier for cancer therapy. Colloids Surf B Biointerfaces. 2015;134:81-7.
60. He Y, Cheng G, Xie L, Nie Y, He B, Gu Z. Polyethyleneimine/DNA polyplexes with reduction-sensitive hyaluronic acid derivatives shielding for targeted gene delivery. Biomaterials. 2013;34(4):1235-45.
● Important investigation that demonstrate successful combination of deshielding and target functions in HA derivatives for gene delivery.

61. Kim EJ, Cho HJ, Park D, Kim JY, Kim YB, Park TG, et al. Antifibrotic effect of MMP13-encoding plasmid DNA delivered using polyethylenimine shielded with hyaluronic acid. Mol Ther. 2011;19(2):355-61.

62. Jang YL, Ku SH, Jin S, Park JH, Kim WJ, Kwon IC, et al. Hyaluronic acid-siRNA conjugate/reducible polyethylenimine complexes for targeted siRNA delivery. J Nanosci Nanotechnol. 2014;14(10):7388-94.
63. Menzel EJ, Farr C. Hyaluronidase and its substrate hyaluronan: biochemistry, biological activities and therapeutic uses. Cancer Lett. 1998;131(1):3-11.
64. Aragona P. Chapter 24 - Hyaluronan in the Treatment of Ocular Surface Disorders. In: Garg HG, Hales CA, editors. Chemistry and Biology of Hyaluronan. Oxford: Elsevier Science Ltd; 2004. p. 529-51.
65. Urbiola K, Sanmartin C, Blanco-Fernandez L, Tros de Ilarduya C. Efficient targeted gene delivery by a novel PAMAM/DNA dendriplex coated with hyaluronic acid. Nanomedicine (Lond). 2014;9(18):2787-801.
66. Campo GM, Avenoso A, Campo S, D'Ascola A, Nastasi G, Calatroni A. Small hyaluronan oligosaccharides induce inflammation by engaging both toll-like-4 and CD44 receptors in human chondrocytes. Biochem Pharmacol. 2010;80(4):480-90.
67. Martens TF, Remaut K, Deschout H, Engbersen JF, Hennink WE, van Steenbergen MJ, et al. Coating nanocarriers with hyaluronic acid facilitates intravitreal drug delivery for retinal gene therapy. J Control Release. 2015;202:83-92.
68. Ohya Y, Takeda S, Shibata Y, Ouchi T, Kano A, Iwata T, et al. Evaluation of polyanion-coated biodegradable polymeric micelles as drug delivery vehicles. J Control Release. 2011;155(1):104-10.
69. Allmeroth M, Moderegger D, Gündel D, Buchholz H-G, Mohr N, Koynov K, et al. PEGylation of HPMA-based block copolymers enhances tumor accumulation in vivo: A quantitative study using radiolabeling and positron emission tomography. Journal of Controlled Release. 2013;172(1):77-85.
70. Hosseinkhani H, Aoyama T, Ogawa O, Tabata Y. Tumor targeting of gene expression through metal-coordinated conjugation with dextran. J Control Release. 2003;88(2):297-312.
71. Heinze T, Liebert T, Heublein B, Hornig S. Functional Polymers Based on Dextran. In: Klemm D, editor. Polysaccharides II. Advances in Polymer Science. 205: Springer Berlin Heidelberg; 2006. p. 199-291.
72. Raemdonck K, Naeye B, Buyens K, Vandenbroucke RE, Høgset A, Demeester J, et al. Biodegradable Dextran Nanogels for RNA Interference: Focusing on Endosomal Escape and Intracellular siRNA Delivery. Advanced Functional Materials. 2009;19(9):1406-15.
● On of the studies that show succesful deliverty siRNAs using dextran as biodegradable cationic dextran nanogels engineered by inverse emulsion photopolymerization.

73. Chang Kang H, Bae YH. Co-delivery of small interfering RNA and plasmid DNA using a polymeric vector incorporating endosomolytic oligomeric sulfonamide. Biomaterials. 2011;32(21):4914-24.
74. Zhang Y, Won C-Y, Chu C-C. Synthesis and characterization of biodegradable hydrophobic–hydrophilic hydrogel networks with a controlled swelling property. Journal of Polymer Science Part A: Polymer Chemistry. 2000;38(13):2392-404.

75. Ochrimenko S, Vollrath A, Tauhardt L, Kempe K, Schubert S, Schubert US, et al. Dextran-graft-linear poly(ethylene imine)s for gene delivery: importance of the linking strategy. Carbohydr Polym. 2014;113:597-606.
●● Interesting work describing the significant impact of linking strategy of lPEI to dextran on the physicochemical characteristics of DNA/polymer complexes, the biocompatibility as well as the transfection efficiency.

76. Thomas JJ, Rekha MR, Sharma CP. Dextran-protamine polycation: an efficient nonviral and haemocompatible gene delivery system. Colloids Surf B Biointerfaces. 2010;81(1):195-205.
77. Jiang G, Min S-H, Oh E, Hahn S. DNA/PEI/Alginate polyplex as an efficientin vivo gene delivery system. Biotechnology and Bioprocess Engineering. 2007;12(6):684-9.
78. Vroman I, Tighzert L. Biodegradable Polymers. Materials. 2009;2(2):307.
79. Santovena A, Alvarez-Lorenzo C, Concheiro A, Llabres M, Farina JB. Structural properties of biodegradable polyesters and rheological behaviour of their dispersions and films. J Biomater Sci Polym Ed. 2005;16(5):629-41.
80. Avérous L, Pollet E. Biodegradable Polymers. In: Avérous L, Pollet E, editors. Environmental Silicate Nano-Biocomposites. Green Energy and Technology: Springer London; 2012. p. 13-39.
81. Bordes P, Pollet E, Avérous L. Nano-biocomposites: Biodegradable polyester/nanoclay systems. Progress in Polymer Science. 2009;34(2):125-55.
● This review highlights the main researches and developments in biopolyester/nanoclay systems.

82. Musyanovych A, Schmitz-Wienke J, Mailander V, Walther P, Landfester K. Preparation of biodegradable polymer nanoparticles by miniemulsion technique and their cell interactions. Macromol Biosci. 2008;8(2):127-39.
83. Luten J, van Nostrum CF, De Smedt SC, Hennink WE. Biodegradable polymers as non-viral carriers for plasmid DNA delivery. J Control Release. 2008;126(2):97-110.
84. Wan Y, Qu X, Lu J, Zhu C, Wan L, Yang J, et al. Characterization of surface property of poly(lactide-co-glycolide) after oxygen plasma treatment. Biomaterials. 2004;25(19):4777-83.
85. Lim Y-b, Choi YH, Park J-s. A self-destroying polycationic polymer: Biodegradable poly (4-hydroxy-L-proline ester). Journal of the American Chemical Society. 1999;121(24):5633-9.
86. Lim Y-b, Kim C-h, Kim K, Kim SW, Park J-s. Development of a safe gene delivery system using biodegradable polymer, poly [α-(4-aminobutyl)-L-glycolic acid]. Journal of the American Chemical Society. 2000;122(27):6524-5.
87. Mahoney MJ, Saltzman WM. Controlled release of proteins to tissue transplants for the treatment of neurodegenerative disorders. J Pharm Sci. 1996;85(12):1276-81.
88. Reul R, Nguyen J, Kissel T. Amine-modified hyperbranched polyesters as non-toxic, biodegradable gene delivery systems. Biomaterials. 2009;30(29):5815-24.

●● Interestingly, this report provides a strong relationship between degree of amine substitution in hyperbranched polyesters carriers and interaction of these polymers with DNA.

89. Madhavan Nampoothiri K, Nair NR, John RP. An overview of the recent developments in polylactide (PLA) research. Bioresource Technology. 2010;101(22):8493-501.
90. Li Z, Huang L. Sustained delivery and expression of plasmid DNA based on biodegradable polyester, poly(D,L-lactide-co-4-hydroxy-L-proline). J Control Release. 2004;98(3):437-46.
91. Liu B, Han SM, Tang XY, Han L, Li CZ. Cervical cancer gene therapy by gene loaded PEG-PLA nanomedicine. Asian Pac J Cancer Prev. 2014;15(12):4915-8.
92. Yang XZ, Dou S, Sun TM, Mao CQ, Wang HX, Wang J. Systemic delivery of siRNA with cationic lipid assisted PEG-PLA nanoparticles for cancer therapy. Journal of controlled release : official journal of the Controlled Release Society. 2011;156(2):203-11.
93. Diao J, Wang H, Chang N, Zhou X-H, Zhu X, Wang J, et al. PEG–PLA nanoparticles facilitate siRNA knockdown in adult zebrafish heart. Developmental biology. 2015;406(2):196-202.
● This study show a novel siRNA knockdown method facilitated by poly(ethylene glycol)-b-poly(d,l-lactide) (PEG-PLA) nanoparticles.

94. Erbetta CDAC, Alves RJ, Resende JM, Freitas RFdS, Sousa RGd. Synthesis and Characterization of Poly(D,L-Lactide-co-Glycolide) Copolymer. Journal of Biomaterials and Nanobiotechnology. 2012;3(2):208-25.
95. Kim D, El-Shall H, Dennis D, Morey T. Interaction of PLGA nanoparticles with human blood constituents. Colloids and Surfaces B: Biointerfaces. 2005;40(2):83-91.
96. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. Journal of controlled release. 2012;161(2):505-22.
97. Sah H, Thoma LA, Desu HR, Sah E, Wood GC. Concepts and practices used to develop functional PLGA-based nanoparticulate systems. International journal of nanomedicine. 2013;8:747.
98. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers. 2011;3(3):1377-97.
99. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids and Surfaces B: Biointerfaces. 2010;75(1):1-18.
100. Lü J-M, Wang X, Marin-Muller C, Wang H, Lin PH, Yao Q, et al. Current advances in research and clinical applications of PLGA-based nanotechnology. 2009.
101. Acharya S, Sahoo SK. PLGA nanoparticles containing various anticancer agents and tumour delivery by EPR effect. Advanced drug delivery reviews. 2011;63(3):170-83.
102. Jain RA. The manufacturing techniques of various drug loaded biodegradable poly (lactide-< i> co</i>-glycolide)(PLGA) devices. Biomaterials. 2000;21(23):2475-90.

103. Sisson AL, Schroeter M, Lendlein A. Polyesters. Handbook of Biodegradable Polymers: Wiley-VCH Verlag GmbH & Co. KGaA; 2011. p. 1-21.
104. Lee YS, Lim KS, Oh J-E, Yoon A-R, Joo WS, Kim HS, et al. Development of porous PLGA/PEI 1.8 k biodegradable microspheres for the delivery of mesenchymal stem cells (MSCs). Journal of Controlled Release. 2015;205:128-33.
105. Liu P, Yu H, Sun Y, Zhu M, Duan Y. A mPEG-PLGA-b-PLL copolymer carrier for adriamycin and siRNA delivery. Biomaterials. 2012;33(17):4403-12.
106. Dailey LA, Wittmar M, Kissel T. The role of branched polyesters and their modifications in the development of modern drug delivery vehicles. J Control Release. 2005;101(1-3):137-49.
107. Suh J, Choy K-L, Lai SK, Suk JS, Tang BC, Prabhu S, et al. PEGylation of nanoparticles improves their cytoplasmic transport. International journal of nanomedicine. 2007;2(4):735.
● This review focuses on designing nanoparticles based on PLGA as drug delivery systems in various biomedical applications such as vaccination, cancer, inflammation and other diseases.

108. Díez S, Miguéliz I, Tros de Ilarduya C. Targeted cationic poly (D, L-lactic-co-glycolic acid) nanoparticles for gene delivery to cultured cells. Cellular and Molecular Biology Letters. 2009;14(2):347-62.
109. Mochizuki M, Hirami M. Structural Effects on the Biodegradation of Aliphatic Polyesters. Polymers for Advanced Technologies. 1997;8(4):203-9.
110. Bhavsar MD, Amiji MM. Development of novel biodegradable polymeric nanoparticles-in-microsphere formulation for local plasmid DNA delivery in the gastrointestinal tract. AAPS PharmSciTech. 2008;9(1):288-94.
111. Palama IE, Cortese B, D'Amone S, Gigli G. mRNA delivery using non-viral PCL nanoparticles. Biomaterials Science. 2015;3(1):144-51.
● A new report to introduce mRNA as mRNA-protamine complex encapsulated poly(ε-caprolactone) (PCL) nanoparticles for the intracellular delivery.

112. Diao HJ, Low WC, Milbreta U, Lu QR, Chew SY. Nanofiber-mediated microRNA delivery to enhance differentiation and maturation of oligodendroglial precursor cells. J Control Release. 2015;208:85-92.
113. Steinbüchel A. Perspectives for Biotechnological Production and Utilization of Biopolymers: Metabolic Engineering of Polyhydroxyalkanoate Biosynthesis Pathways as a Successful Example. Macromolecular Bioscience. 2001;1(1):1-24.
114. Loh XJ, Ong SJ, Tung YT, Choo HT. Incorporation of poly[(R)-3-hydroxybutyrate] into cationic copolymers based on poly(2-(dimethylamino)ethyl methacrylate) to improve gene delivery. Macromol Biosci. 2013;13(8):1092-9.
115. Li Z, Loh XJ. Water soluble polyhydroxyalkanoates: future materials for therapeutic applications. Chemical Society reviews. 2015;44(10):2865-79.

116. Kilicay E, Erdal E, Hazer B, Turk M, Denkbas EB. Antisense oligonucleotide delivery to cancer cell lines for the treatment of different cancer types. Artificial cells, nanomedicine, and biotechnology. 2015:1-11.
117. Rehm BH. Bacterial polymers: biosynthesis, modifications and applications. Nat Rev Microbiol. 2010;8(8):578-92.
118. Oppermann-Sanio FB, Steinbuchel A. Occurrence, functions and biosynthesis of polyamides in microorganisms and biotechnological production. Naturwissenschaften. 2002;89(1):11-22.
119. Salahuddin N, Elbarbary A, Allam NG, Hashim AF. Polyamide-montmorillonite nanocomposites as a drug delivery system: Preparation, release of 1,3,4-oxa(thia)diazoles, and antimicrobial activity. Journal of Applied Polymer Science. 2014;131(23):n/a-n/a.
120. Guo J, Cheng WP, Gu J, Ding C, Qu X, Yang Z, et al. Systemic delivery of therapeutic small interfering RNA using a pH-triggered amphiphilic poly-L-lysine nanocarrier to suppress prostate cancer growth in mice. Eur J Pharm Sci. 2012;45(5):521-32.
● A study to show the effecet of a biocompatible nanoparticle cntaining poly-L-lysine in delivery of therapeutic small interfering RNA in order to suppress prostate cancer growth.

121. Zhou D, Li C, Hu Y, Zhou H, Chen J, Zhang Z, et al. Glycopolymer modification on physicochemical and biological properties of poly(L-lysine) for gene delivery. Int J Biol Macromol. 2012;50(4):965-73.
122. Bajaj I, Singhal R. Poly (glutamic acid)--an emerging biopolymer of commercial interest. Bioresour Technol. 2011;102(10):5551-61.
123. Hattori Y, Nakamura A, Arai S, Nishigaki M, Ohkura H, Kawano K, et al. In vivo siRNA delivery system for targeting to the liver by poly-l-glutamic acid-coated lipoplex. Results Pharma Sci. 2014;4:1-7.
124. Peng SF, Yang MJ, Su CJ, Chen HL, Lee PW, Wei MC, et al. Effects of incorporation of poly(gamma-glutamic acid) in chitosan/DNA complex nanoparticles on cellular uptake and transfection efficiency. Biomaterials. 2009;30(9):1797-808.
125. Li LC, Deng J, Stephens D. Polyanhydride implant for antibiotic delivery--from the bench to the clinic. Adv Drug Deliv Rev. 2002;54(7):963-86.
126. Kumar N, Langer RS, Domb AJ. Polyanhydrides: an overview. Adv Drug Deliv Rev. 2002;54(7):889-910.
127. Azevedo C, Saiardi A. Functions of inorganic polyphosphates in eukaryotic cells: a coat of many colours. Biochem Soc Trans. 2014;42(1):98-102.
128. Liu J, Huang W, Pang Y, Yan D. Hyperbranched polyphosphates: synthesis, functionalization and biomedical applications. Chemical Society reviews. 2015;44(12):3942-53.
129. Chaubal MV, Gupta AS, Lopina ST, Bruley DF. Polyphosphates and other phosphorus-containing polymers for drug delivery applications. Crit Rev Ther Drug Carrier Syst. 2003;20(4):295-315.

130. Wang YC, Yuan YY, Du JZ, Yang XZ, Wang J. Recent progress in polyphosphoesters: from controlled synthesis to biomedical applications. Macromol Biosci. 2009;9(12):1154-64.
131. Ditto AJ, Reho JJ, Shah KN, Smolen JA, Holda JH, Ramirez RJ, et al. In vivo gene delivery with L-tyrosine polyphosphate nanoparticles. Mol Pharm. 2013;10(5):1836-44.
● The first report in the potential use of controlled-release nanoparticles formulated from an amino acid based polymer as an in vivo nonviral vector for gene therapy.

132. Huang X, Shen S, Zhang Z, Zhuang J. Cross-linked polyethylenimine-tripolyphosphate nanoparticles for gene delivery. Int J Nanomedicine. 2014;9:4785-94.