Viral- and Non-viral-Based Hybrid Vectors for Gene Therapy

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Manohar Mahato, Giridhara R. Jayandharan, and Praveen Kumar Vemula

4.1 Introduction

Paradigm changing recent developments in the field of nanotechnology offer the platform where Physicochemical and biological properties of metallic and nonmetallic molecules can be modulated for the wide range of applications in several areas of communications, basic sciences, engineering, medicine, robotics, etc. [1–4]. Since the introduction of the term nanotechnology, various modifications have been implemented to develop novel variants of nanoparticles with diverse properties [5, 6]. Specifically, nanoparticles are in the size range of 5–100 nm and possess high surface area to volume ratio which renders them to bind molecules with bi-specific conjugate or specific targeting peptides [7]. Based on the composition of nanoparticles, they can be categorized as polymeric (synthetic or natural polymers), Q-dots, nanoemulsions, ceramic (silica) particles, metallic (gold, silver, iron oxide), liposomes, and graphene [8]. Accordingly, nanoparticles exhibit specific optical, magnetic, chemical, and physical properties that lead to their application in various biomedical applications such as in vivo imaging, tissue-specific drug delivery, etc. [9–11]. The major advantage of nanoparticles containing tissue-specific moiety as a delivery vehicle is the ability to bypass side effects associated with therapeutics such as antibiotics or chemotherapeutic agents [12]. Thus, such properties make them useful for both vaccination and therapeutic strategies to circumvent an immune response or for gene delivery, respectively [13]. Delivery of a normal copy of genes inside the cells is a promising approach for the treatment of various genetic or acquired disorders and is also called as replacement gene therapy. Viruses are the

M. Mahato · P. K. Vemula (✉)
Institute for Stem Cell Biology and Regenerative Medicine (inStem), Bangalore, India
e-mail: praveenv@instem.res.in

G. R. Jayandharan
Department of Biological Sciences and Bioengineering, IIT-Kanpur, Kanpur, UP, India

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potential candidates for delivering therapeutic genes inside the cells with higher efficiency; to achieve the therapeutic gene expression, various recombinant vectors have been used including adenovirus, retro-/lentivirus, and adeno-associated virus (AAV) [14]. Till date, >2300 clinical trials for gene therapy are going on worldwide out of which 70% are using viral vectors, but none of them have reached desired therapeutic endpoints [14–16]. The primary objective of gene therapy is to maintain stable transgene expression, but the major hurdle for gene therapy is host immunity which hinders persistent high levels of transgene expression [17]. Since the viruses are a coating of proteins, thus they are recognized as foreign bodies by host immune system and lead to their activation. The host immunity serves as a major side effect for gene therapy through viral vectors. Recently, some reports showed using the combination of nanoparticle and viral vectors to optimize therapeutic delivery, for example, viral vectors encapsulated in nanomaterials rescued gene therapy vectors from adverse immunity events [18, 19]. Taking a cue from these kinds of studies, this chapter summarizes the data available on hybrid vector-based delivery systems which consist of nanoparticle and viral vectors, the strategies to enhance the potential of the hybrid system, and their advantages.

4.2 Concept of Gene Therapy

The technique of delivering a therapeutic gene into host cells to ameliorate the genetic disorder or acquired disease is called gene therapy. The gene therapy can be classified into two categories as (1) somatic cell gene therapy, limited to individual, and (2) germline gene therapy – modified gene is inheritable. Due to ethical issues, insufficient knowledge, and risk to future generations, germline gene therapies are prohibited in many countries. Thus, most of the gene therapy programs have been focused on somatic gene therapy. Since its discovery in 1980, the first clinical trial was established in 1990 using retrovirus as a vector for functional adenosine deaminase [20]. Till date, >2300 clinical trials have been conducted using various viral vectors in different parts of the world [14]. As advancement in the field of viral vectors, alternative approaches have been implemented and showed promising potential by knocking down the mutated gene (suicide gene therapy) or editing faulty gene (nuclease-mediated gene editing) to reach therapeutic endpoint [21]. These delivery systems have been used in two distinct modes: (1) ex vivo, where viral vector with therapeutic gene is transduced in recipient cells (e.g., hematopoietic cells) and then transduced cells are introduced into the host body, and (2) in vivo, where the viral vectors are administered directly into host body [22]. The choice of approach is mainly based on disease and target tissue to be treated from gene delivery. An ideal vector should exhibit sustained transgene expression and tissue specificity with low immunogenicity for higher therapeutic efficacy through critical designing of viral vectors; they have been used with fair success for various genetic disorders like cystic fibrosis [23], hemophilia [24], Leber's congenital amaurosis [25], and various severe combined immunodeficiency (SCID) [26, 27]. Currently, three kinds of viruses, i.e., adenovirus, retrovirus/lentivirus, and adeno-associated

virus (AAV), have been employed as vectors for delivering therapeutic genes to the desired cells [14]. These vectors have shown promising results and success in gene therapy up to some extent. Thus, there is a scope for further advancement for improving the therapeutic efficacy.

4.3 Viral and Non-viral Hybrid Vectors

Since the 1980s, both viral and non-viral vectors (synthetic) have been developed to overcome the limitations associated with both delivery systems for making gene therapy a viable technology in clinics (Fig. 4.1). Although viral vectors have a high transduction efficacy for DNA, they are immunogenic. On the contrary, non-viral vectors have low transfection efficacy than viral vectors, but generally, they have low immunogenicity (as they have been designed from biocompatible material). Thus, to leverage the advantages from both types of vectors, hybrid vectors were developed by the combination of both vectors to achieve higher gene delivery efficacy than individual vector alone with minimal side effects (i.e., immunogenicity). In an attempt to develop hybrid vectors, viral vectors (AAV, adenovirus, retro-/lentivirus) have been encapsulated within synthetic materials such as liposomes, dendrimers, and hydrogels (Figs. 4.3 and 4.4). Some of hybrid vectors that have shown significant efficacy in delivering genes are listed in Table 4.1. Among all the viral vectors, adenovirus showed promising potential for development of hybrid vector systems as they were able to target tumor tissues efficiently [28–32]. Moreover, it can function effectively with different non-viral vectors (Table 4.2) such as alginate

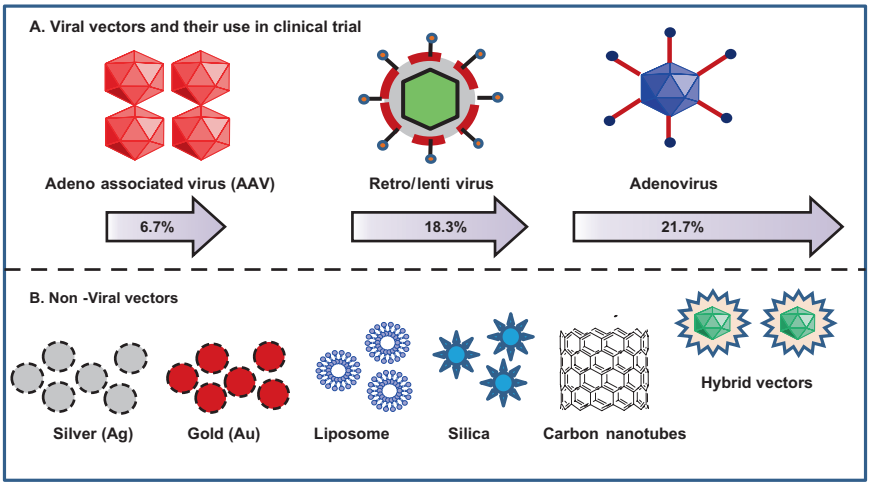


Fig. 4.1 Various nanoparticle and gene therapy vectors. (**a**) Viral vectors, e.g., adenovirus, adeno-associated virus, and lentivirus, which have been dominantly used in clinical trials. (**b**) Non-viral vectors and metallic and nonmetallic nanomaterials have been developed with targeting capability

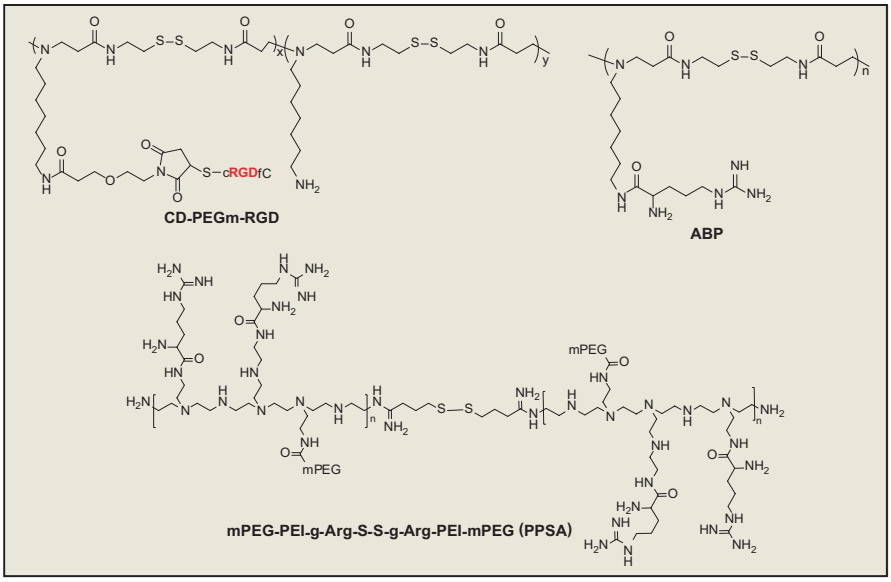
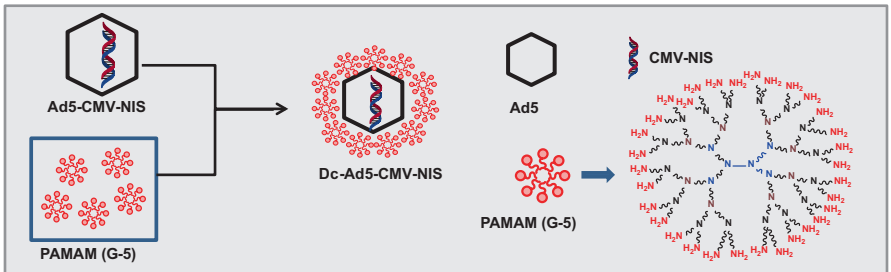


Fig. 4.2 Chemical structure of bioreducible polymer used for coating over adenoviruses to develop various hybrid vectors



Experiments	Naked adenovirus (Ad5-CMV-NIS)	Dendrimer coated adenovirus (Dc-Ad5-CMV-NIS)
In vitro		
➢ HuH7 (CAR-positive cells)	70-fold transduction	70-fold transduction
➢ U87 MG (low CAR-positive cells)	Low transduction	5.5-fold transduction
➢ SKOV-3 (CAR-negative cells)	No transduction	22-fold transduction
➢ Adenovirus neutralizing antibodies (IgG)	Rapid neutralization	Effective protection
In vivo		
➢ Virus biodistribution (hepatic pooling)	High	~70% lower
➢ hepatic NIS mRNA expression	High	~16-fold lower
➢ tumoral iodide accumulation	3.5% ID/g	13.5% ID/g
➢ NIS mRNA expression in tumor cell	2.5 fold increase	10-fold increase

Fig. 4.3 Schematic representation of formation of PAMAM-G5-coated adenovirus and their efficacy in in vitro and in vivo

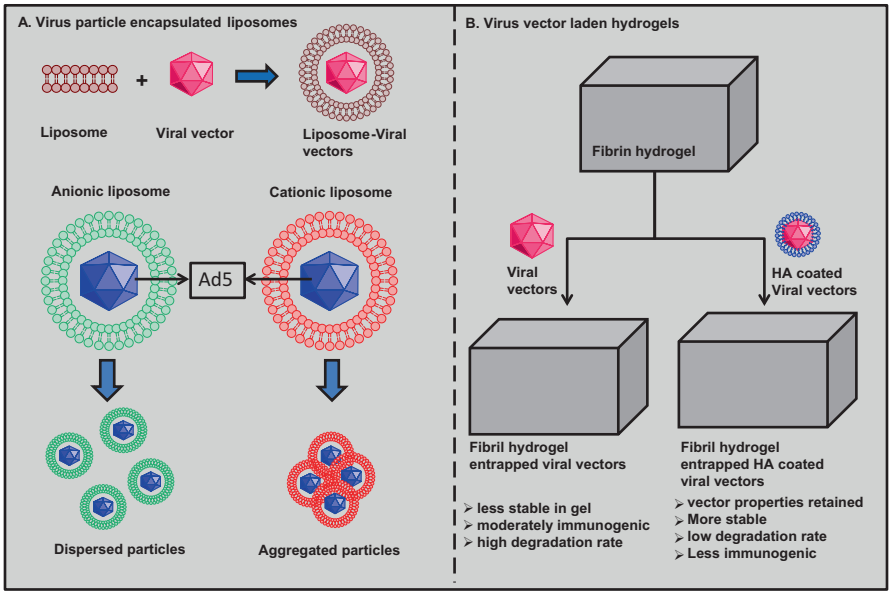


Fig. 4.4 Hybrid viral nanoparticles. The combination of viral vectors and nanomaterials offers many advantages such as delivery of multiple payloads, targeting ability to specific tissue, and escape from host immune system. The schematic representation of the advantages of hybrid vectors. (**a**) Variations between the cationic liposomally bound viral particles and anionic liposomally bound nanoparticles. (**b**) Viral particles encapsulated in fibrin hydrogels. Difference between naked viral particles and hydroxyapatite (HA)-coated viral particle-loaded in fibrin hydrogels is highlighted

beads [33], chitosan [34, 35], chitosan-PEG-folate conjugate [36], polyethyleneimine [37, 38], etc. To develop tissue targeting hybrid vectors, conjugation of moieties like arginine graft [39], RGD [40], and Herceptin [41] and even surface charge modification [38, 42] have shown precise targeting by vectors (Fig. 4.2 and Table 4.3). The arginine-grafted bioreducible polymers (ABP) were synthesized, and hybrid vectors were developed with adenovirus to overcome the immune response from the host with minimal cytotoxicity. In vitro results showed after electrostatic coating of ABP over adenovirus resulted in enhancement of six-fold transduction efficiency in coxsackievirus and adenovirus receptor (CAR)-negative cells as compared to naked Ad vectors [39]. These results suggested after cationic polymer coating, hybrid vectors internalize within the cell through CAR-independent pathway. Moreover, ABP-Ad vector showed 83.1% of transduction efficiency in the presence of 30% serum in A549 cells, while naked Ad vectors showed 47.49% efficiency. Further, an innate immune response was evaluated after treating RAW264.7 macrophage cells with naked Ad and ABP-Ad vectors. Pro-inflammatory cytokine IL-6 release was significantly reduced after treatment with ABP-Ad (38.57 ± 0.5 pg/mL) as compared to naked Ad (70.35 ± 0.5 pg/mL). These results strongly suggested that shielding of the viral proteins with cationic polymers can enhance

Table 4.1 List of nanomaterial-coated hybrid viral vectors that have been tested either in vitro or in vivo

S. No.	Vector	Nanomaterial	Transgene	Assay endpoint	Reference
1.	Adenovirus	PAMAM-G5	Sodium-iodide symporter	^{123}I Scintigraphy^{+} (radiovirotherapy)	[45]
2.	Lentivirus	Fibrin hydrogel (+/− hydroxyl apatite)	Luciferase	Bioluminescence	[53]
3.	Lentivirus	Collagen hydrogel (+/− hydroxyl apatite)	Luciferase	Bioluminescence	[52]
4.	AAV	Elastin-like polypeptide (ELP)	GFP	In vitro transduction	[54]
5.	AAV	Heparin-coated superparamagnetic iron oxide	GFP	In vitro transduction	[55]
6.	AAV	Glyceraldehyde tag	GFP	In vitro transduction	[86]
7.	AAV	Elastin-like polypeptide + poly(ε-caprolactone)	GFP	In vitro transduction	[87]
8.	AAV	Polyethylene glycol (PEG)	GFP	In vitro transduction	[88]

Table 4.2 Hybrid vectors composed of polymer/Ad

Polymer	Adenovirus	Efficacy
Reducible PEI	RdB/shMet	Enhanced transduction efficiency – increased viral entry and production in vitro [38]
Bile acid-conjugated PEI	KOX	Enhanced antitumor therapeutic efficacy and antiangiogenic effect than cognate control virus in vitro and in vivo [37]
PNLG	Δ B7-U6ShIL8	Preserved Ad's biological activity at 37 °C – significantly enhanced antitumor efficacy than either Δ B7-U6ShIL8 or Δ B7-U6ShIL8/ABP in HT1080 and A549 tumor models in vitro and in vivo [29]
PPSA	DWP418	Enhanced transduction efficiency and antitumor efficacy than Ad/ABP in vitro and in vivo [30]
PAMAM	E1/AFP-E3/NIS	Synergistic therapeutic effect by combining oncolytic Ad and therapeutic dose of ^{131}I in vitro and in vivo [39]
PEG	Ad-GL	More potent antitumor effect and less hepatotoxicity by 20-kDa PEGylated oncolytic Ad than 5-kDa PEGylated oncolytic Ad in vitro and in vivo [38]
ABP	YKL-1001	Increased blood circulation time and safety profiles – enhanced antitumor therapeutic efficacy than cognate control in hepatoma xenograft model in vitro and in vivo [35]

Table 4.3 Modified polymers used for development of targeting hybrid vectors

Polymer	Targeting ligand	Oncolytic adenovirus	Targeted receptor/cell	Efficacy
Chitosan-PEG-FA	Folic acid	HmT	Folate receptor overexpressed cancer	Folic acid-mediated antitumor efficacy of Ad/polymer is higher than EPR-mediated delivery [32]
CD-PEG-cRGD	cRGD	Δ B7-U6ShIL8	αβ integrin positive cancer	Greater antitumor efficacy than naked Ad in A549 lung orthotopic model [36]
PEG-HER	Herceptin	DWP418	Her2/neu overexpressed cancer	Her2 targeted specific transduction and antitumor efficacy [37]
PAMAM-GE11	GE11	E1/AFP-RSV/NIS	EGFR-positive cancer	EGFR targeted specific antitumor efficacy by combination of polymer-coated Ad and ^{131}I [46]

circulation period for hybrid vectors and reducing innate immune response. In a similar study, a cationic biodegradable polymer, methoxy poly(ethylene glycol)-b-poly{N-[N-(2-aminoethyl)-2-aminoethyl]-L-glutamate} [PNLG], was synthesized, and hybrid vectors were developed with adenoviruses [29]. The transduction efficiency of developed PNLG-Ad vector was compared to ABP-Ad vectors in vitro and in vivo. The PNLG-Ad vectors exhibited high stability at 37 °C and pharmacokinetics due to the formation of smaller particle size (~130–140 nm), while ABP-Ad vector formed 400–1300 nm size particles. The tumor growth was reduced in various xenograft models such as 57.5% (HT1080)/47.0% (A549), whereas ABP-Ad showed reduction up to 24.8% (HT1080)/16.4% (A549). The innate immune response was also evaluated by quantifying the IL-8 and vascular endothelial growth factor (VEGF) released after treatment with ABP-Ad, PNLG-Ad, and naked Ad vectors. The results showed significant inhibition of IL-8 or VEGF secretion, 76.6% or 79.7%, respectively, on treatment with PNLG-Ad while 47.7% or 60.7% with ABP-Ad. Moreover, systemic administration of PNLG-Ad vector exhibited a 1229-fold increase in tumor to the liver ratio as compared to naked Ad. These studies revealed that biophysical property of hybrid vectors such as particle size and surface charge plays a crucial role in their therapeutic efficacy. Similarly, several reports have been published using cationic bioreducible polymers (exclusively polyethylenimine) [37, 38] and mPEG-PEI-g-Arg-S-S-Arg-gPEI-mPEG [30] for the development of hybrid vectors.

After successful development of hybrid vectors which can internalize inside the cell through CAR-independent pathway without triggering the immune response, researchers focused on developing hybrid vector with targeting ability. In an attempt to develop targeting hybrid vector against folate receptor (FR)-positive cancer, adenovirus was electrostatically complexed with chitosan [36]. Then, polyethylene

glycol (PEG)/folic acid (FA) or PEG-FA was chemically conjugated to the surface of chitosan-Ad to develop various nanocomplex such as chitosan-Ad, chitosan-PEG-Ad, chitosan-FA, and chitosan-PEG-FA-Ad. The vectors consisting of FA on the surface (chitosan-FA-Ad, chitosan-PEG-FA-Ad) exhibited significant selectivity against folate receptor-positive cells (HeLa and KB cells) and showed cell viability up to ~45% in KB and HeLa cells while ~70–80% in FR-negative cells (U343 and A549 cells). Systemic administration of chitosan-PEG-Ad and chitosan-PEG-FA-Ad significantly increased the blood circulation time after 24 h of injection, resulting in 9.0-fold and 48.9-fold increase, respectively, as compared to naked Ad. Moreover, these hybrid vectors showed ~75% decrease in generation of adenovirus-specific neutralizing antibodies in mice when treated with chitosan-PEG-FA-Ad as compared to naked Ad. The administration of chitosan-PEG-FA-Ad exhibited 378-fold reduction in liver tissues and 285-fold increase in tumor tissue as compared to naked Ad; hence the hybrid vector was able to enhance the tumor-to-liver ratio. The targeting hybrid vector exhibited 52.8% inhibition of tumor growth as compared to naked Ad. Thus, conclusively chitosan-PEG-FA-Ad showed promising potential for further development of targeting hybrid vectors in terms of efficacy and safety. To develop targeting vectors against endothelial cells of tumor capillaries and neointimal tissues, a bioreducible cationic polymer CD was conjugated to cyclic RGD peptide (Fig. 4.2). These tissues inherently overexpresses αvβ3 and αvβ5 integrin proteins which selectively bind to RGD peptides. Two hybrid variants were synthesized with different molecular weights of PEG chains, viz., PEG_{500} and PEG_{2000} to generate CD-PEG_{500}-RGD-Ad and CD-PEG_{2000}-RGD-Ad [40]. The results showed RGD-tethered polymer-coated hybrid vectors were specifically killing the cancer cells having integrin protein on cell membrane, irrespective of CAR. The CD-PEG_{500}-RGD-Ad hybrid vector was efficiently able to express shRNA against IL-8 mRNA. There was significant reduction of IL-8 expression in cancer cells was observed as compared to naked Ad, such as 79.6% decrease in HT1080 and 77.2% decrease in MCF7 cells. Further, exploiting the cell surface biomarker as a target which is overexpressed on cancer cells, various potential ligands have been investigated. Her2/neu is widely known as human epidermal growth factor 2 receptor and overexpressed in 20–30% of breast cancer patients. This receptor plays an important role as an oncogene in cancer cells. Drugs which target these receptors like trastuzumab and lapatinib are in clinical use; trastuzumab (Herceptin), a monoclonal antibody specific for Her2/neu, is also being used widely for treatment of both early and metastatic breast cancer [43, 44]. To develop Her2/neu targeting hybrid vector, adenovirus (Ad) was chemically conjugated with bioreducible PEG chain, and Herceptin was tethered terminally, HER-PEG-Ad [41]. Specificity and CAR-independent cellular uptake of these Herceptin-conjugated hybrid vectors were evaluated in vitro using Her2-positive (MDA-MB435, SK-OV3, and MDA-MB231) and Her2-negative (SK-Her1 and HeLa) cells. Further, innate response and stability of the HER-PEG-Ad were evaluated after systemic administration in BALB/c mice. The results showed after administration, IL-6 secretion level was found to be 77 pg/mL, 14 pg/mL, 411 pg/mL, and 46 pg/mL for HER-PEG-Ad, PEG-Ad, naked Ad, and PBS, respectively. After 1 h of administration, HER-PEG-Ad and PEG-Ad

were six-fold higher than naked Ad in blood circulation. The ligand-modified hybrid vector showed significant higher targeting ability for tumor in xenograft model, and HER-PEG-Ad showed 10^{10}-fold increase in tumor-to-liver ratio with minimal hepatic toxicity. These reports suggest that the development of hybrid systems from nanomaterial-coated viral vector using nonpathogenic viruses like AAV serves as excellent candidates for higher efficacy with minimal side effects.

4.4 Dendrimer-Coated Virus Particles

In a recent study, the hybrid vector (as shown in Fig. 4.3) was developed for gene transfer in liver cancer xenograft model from adenovirus coated with poly(amidoamine) dendrimer generation 5 (PAMAM-G5) [45]. The transduction efficacy and tissue tropism of coated adenovirus particles (Ad5-CMV/NIS) which consist of hNIS transgene (sodium-iodide symporter) were tested by radioactive iodine isotope (^{123}I) scintigraphy. The in vitro results have shown a significant decrease in antibody-mediated neutralization and increase in the CAR-negative cell (extent in adenovirus infection). Further, when this hybrid vector was administered in mice, it showed sustained transgene expression and reduction in tumor size as well. The study showed such delivery systems using adenovirus hybrid vectors indicate high therapeutic potential. Moreover, to incorporate targeting ability to the dendrimer-based hybrid vectors, dendrimer was conjugated to the peptide as a ligand specific for epidermal growth factor receptor (EGFR), PAMAM-GE11 [46]. In this study, PAMAM-G2 and PAMAM-G5 were used, but PAMAM-G2-GE11 showed better efficiency due to improved covering of adenoviral surface epitopes by smaller diameter of dendrimers. This hybrid vector also showed CAR-independent cellular uptake with low hepatic accumulation as well as an increase in transduction efficiency over tumor cells in the xenograft model.

4.5 Virus Particles Encapsulated Liposomes

Viral gene therapy holds great potential in treating cancer using oncolytic replication-selective viruses (OVs) as they selectively replicate within cancer cells and causes apoptosis [47]. The use of OV-based gene therapy showed significant alleviation of cancer in human clinical trials even with advanced stages of cancer [48]. However, their efficacy has been limited by rapid clearance through reticuloendothelial (RE) system in liver and neutralization by antibodies which affect their distribution into the tumor cells [49]. To overcome the issue of neutralization of OV by antibodies, Yotnda P. et al. have encapsulated adenovirus vectors in bilamellar cationic liposomes consists of DOTAP (1,2-dioleoyloxypropyl)-N,N,N-trimethylammonium chloride) and cholesterol [50] (Fig. 4.4a). This hybrid vector was able to efficiently transfect the cells which either lacks adenoviral receptors or in the presence of receptor, as compared to naked adenovirus. However, their clinical application was hindered due to systemic toxicity, low targeting efficacy, and poor serum stability. To address these issues, adenoviral vectors (adenovirus 5, Ad5) were encapsulated in

anionic bilamellar liposomes composed of phosphatidylcholine, phosphatidylethanolamine, inositol phosphatides, cholesterol, PEG-2000, and nontoxic lecithin (Fig. 4.4a) [51]. These anionic liposome-encapsulated adenoviral-based hybrid vectors have shown superior transfection efficacy in cancer cells than naked Ad5 and were able to administer repeatedly without any immunogenic response in vivo. Moreover, the anionic liposomal virus particles have shown stability for 32 h as a monodisperse solution, while cationic liposomal virus particle got aggregated within a couple of hours (Fig. 4.4A). The anionic liposomal-based encapsulated viral particles have shown promising results for further use in clinical application [51].

4.6 Virus Vector-Laden Hydrogels

In a study to develop a better transduction profile with lentiviruses, lentiviruses were encapsulated in hydrogels composed of collagen and hydroxyapatite [52]. The effect of material used for hydrogel formation and their degradation kinetics for transgene expression was evaluated both in vitro and in vivo. The encapsulated lentivirus showed ~80% of transfection efficiency in invasive C6 glioma cells. Further, the virion release and cell migration from the surrounding tissue was depending on the composition of collagen hydrogel (0.05%, 0.15%, 0.3%). While the efficacy of lentivirus loaded in hydroxyapatite containing collagen-gels was marginal (~33% increase in luciferase gene expression) as compare to only collagen-containing gels in an animal model (CD-1 male mice). Similarly, another study was carried out using fibrin and hydroxyapatite hydrogel encapsulated lentiviruses for localized vector transduction in CD-1 mice (Fig. 4.4b), but this strategy did not affect the virus infectivity or their cellular infiltration [53]. To develop high-performance delivery systems, researchers have used AAV vectors combined with elastin-like polypeptides (ELP) and evaluated for their infectivity on human neural stem cells (NSCs) and murine fibroblasts (NIH3T3) [54]. This study was carried out using AAV variant r3.45 which showed a significant increase in transduction efficacy when conjugated to ELP as compared to control groups. The results showed potential use of these hybrid vectors in NSCs for the treatment of various neurodegenerative disorders. To maximize the AAV contact with tissue for efficient and sustained gene transfer, AAVs were encapsulated in a nanofiber scaffold composed of ELP and poly(ε-caprolactone) (PCL) through electrospinning [18]. The super paramagnetic iron oxide nanoparticles (SPION) were coated with heparin and combined with AAV variant r3.45 which showed enhanced gene delivery in different types of cell lines, e.g., HEK293T and PC12 cell lines [55]. Even a short incubation period of <180 min was sufficient in transducing the target cells with the same efficiency achieved with conventional 24-hour incubation of naked virus. Moreover, the magnetically driven AAV transduction improved some of the critical phenotypes such as the neurite extension and expression for nerve growth factor in PC12 cells. These reports suggest that the hybrid vectors have several advantages over the convention delivery vectors, but its development requires exhaustive understanding related to host immune response, tissue specificity, and kinetics of nanoparticle-vector hybrid delivery under in vivo settings.

4.7 Challenges

The cellular uptake of nanoparticles in a large quantity inside the host cells generates concentration gradient across vascular endothelium which leads to inhibition of further uptake [56]. Moreover, this phenomenon stimulates the residential monocytic-phagocytic system and results in an aberrant distribution of nanoparticles (Fig. 4.5). However, there are various properties of hybrid nanoparticles such as size, surface charge, stability, and route of delivery which decide the efficiency of these vectors inside the cell [57, 58]. Under in vivo condition, the interaction of natural multivalent biomolecules in blood circulation and viral vectors generates the immune response and has been described below [59–61]. Many reports are suggesting that the generation of immune response associated with administering vaccines and drug delivery vehicles [10]. The extent of innate response mainly depends on targeted tissue (e.g., skin, lungs, gut), as each of tissue having a different number of residential immune cells [61, 62]. After cellular uptake, hybrid vectors got fragmented and stimulate either innate or adaptive immune response through a cascade of events initiating from antigen generation by antigen-presenting cells (APCs) to

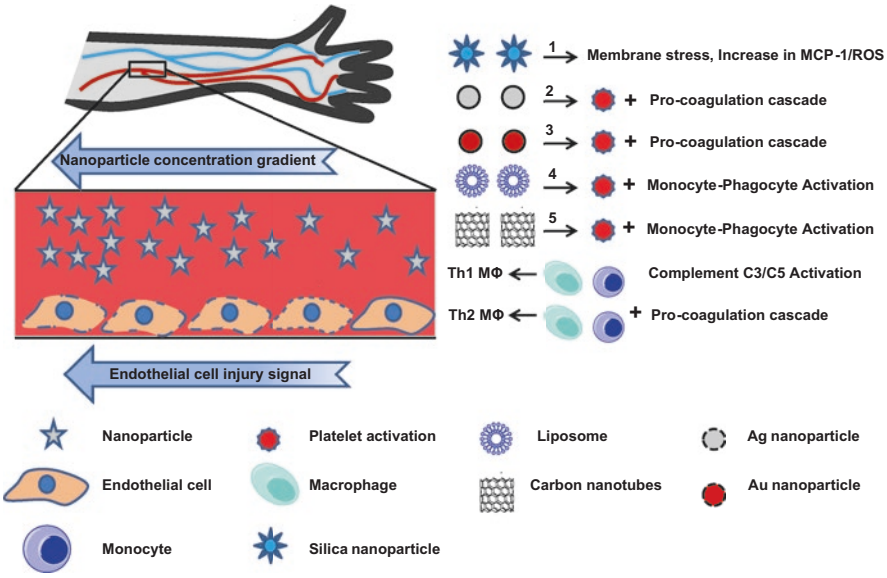


Fig. 4.5 Innate immune response against nanoparticles. The entry of nanoparticles through endothelial cells is a primary event that triggers a cascade of signals toward nanoparticles or their fragments [75]. Innate immune response toward them differs substantially as it depends on the physical characteristics of nanoparticle such as size, shape, charge, and associated ligand/peptide molecules as well as route of entry [76–78]. The invasion of nanoparticle through endothelial cells leads to disruption of endothelial membrane and eventually activates vascular system along the nanoparticle concentration gradient. Events 1–5 show five different nanoparticles, i.e., silica, silver, gold, liposome, and carbon nanotubes, and the innate response observed against them. Macrophage-mediated phagocytosis of nanoparticle/ fragment involves multiple events including macrophage migration and differentiation in response to cytokines/chemokines that activates Th1Mϕ /Th2Mϕ cells [79–81]

Table 4.4 Immune response reported with various different nanoparticle formulations

S. No.	Nanomaterial	Primary events lead to immune response
1.	Gold nanoparticles	Platelet activation, plasma membrane disruption [60, 89]
2.	Silver nanoparticles	Induce cytotoxicity to endothelial cells, pro-inflammatory cytokine, chemokine production, NF-KB pathway activation, free radical production [64]
3.	Metal oxide nanoparticles	Chemokine receptor molecule (type 4, CXCR4), adhesion molecule expression levels [90]
4.	Silica nanoparticles	Nitric oxide and peroxynitrite production; upregulation of ICAM1, VCAM1, IL-8, and IL-6; NF-KB activation [91]; reactive oxygen species generation; apoptotic signal molecule generation and transcription factor upregulation; release of tissue factor, IL-6, IL-8, MCP-1, and ROS [92]
5.	Carbon nanotubes	Complement-mediated opsonization, C3/C5 and membrane attack complex formation [93] Endothelial membrane leakage [94] Platelet activation and aggregation, degranulation, ATP release [95] Oxidative stress induction, cytokine production (TNF-α), IL-1β, and IL-8 [63, 96–99] Inflammation [100–104]
6.	Dendrimers	Endothelial cytotoxicity, endotoxin-induced procoagulant activity [98, 105, 106]
7.	Liposome	Expression of macrophage maturation marker and polarization of monocyte Inhibition of macrophage migration [107] Endothelial cell cytotoxicity [79]
8.	Cationic lipids (RPR206252)	TNF-α, IL-1β, IL-6, IFN-γ production NF-KB activation, TLR-2 and NLRP3 activation [108]
9.	Polystyrene latex particle	Platelet activation and aggregation, upregulation of adhesion receptor [109]
10.	1,3-β-Glucan chitosan shell with poly(lactide-co-glycolide)	Reactive oxygen species, reactive nitrogen species, pro-inflammatory cytokine secretion, increased expression of TNF-α and IFN-γ [110]
11.	Perfluorocarbon emulsion	Complement system activation [111, 112]

exocytosis or leading to cellular apoptosis [63]. The invasive property of hybrid vectors which causes endothelial cell injury and malfunction acts as the first sign for toxic effects on vascular system (Fig. 4.5) [64]. The immune response associated from various nanoparticle has been summarized in Table 4.4, but the overlap between the response generated is also frequently observed. In Fig. 4.6, detailed schematic representation of a possible number of events which lead to adaptive immunity in the presence of nanoparticles has been described. The nanoparticle antigens are captured by immature dendritic cells from closest lymph nodes which lead to the activation of T-cell differentiation and stimulating B cell as well [65]. Several inflammatory cytokines (TNF-α and IL-1β) and co-stimulatory receptor ligands CD80 (B7-1) and CD86 (B7-2) are responsible for activation and

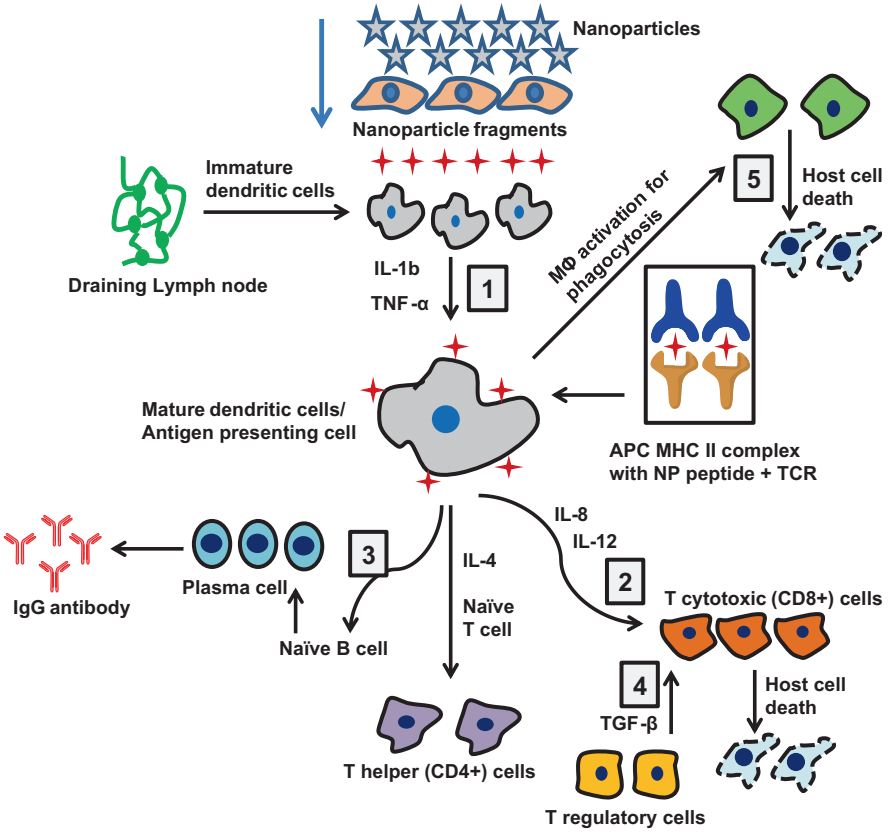


Fig. 4.6 This schematic representation depicts the adaptive immunity observed against the nanoparticles. (1) Dendritic cells act as a link between innate and adaptive immune system and regulate their cross activation through several signals (MHCI/II-peptide complex, CD80-CD80L, etc.) [66–69]. Movement of DCs bearing the peptide/MHC complex toward lymph nodes [82] is a critical factor that determines the magnitude of this activation. (2) In response to MHCI/II complex, cytokines (IL-4, IL-6, IL-12, TGF-β) and chemokines are released by naïve T cells that further activate downstream effectors such as the residential macrophages/monocytes which capture and destroy nanoparticle containing host cells. (3) Nanoparticle interaction with adaptive immune cells/molecules that leads to activation of host dendritic and cytotoxic T-cell population [83]. Some reports suggested that the nanoparticle coated with peptide ligand can also activate B cell and generate antibodies. (4) Nanoparticle-mediated response through T cell or B cell has been bypassed through activation of T-regulatory cells and suppression of pro-inflammatory molecules (IL-2, IL-6, TNF-α, etc.). (5) Macrophage activation and differentiation by dendritic cells and nanoparticle phagocytosis leads to the expulsion of nanoparticle from the host cells [84, 85]

functionalization of DCs with antigen [66]. These activated DCs perform a cascade of signals along with MHC class I and II molecules to naive T cells having T-cell antigen receptor (TCR) [67]. Co-stimulatory signals CD80/86 which are generated from APCs interact with CD28 (T-cell receptor), and simultaneously secretion of cytokines (IL-12, IL-14, IL-16, TGF-β) also takes place which stimulates naive T

cells to differentiate into Th1, Th2, or Th17 cells. The antigen functionalization involves MHC class II loading pathway [68] which leads to the generation of a limited number of CD8+ T cells, and thus antigens can only be presented to only specific groups of DCs in the spleen or lymph nodes [69]. These pathways are suggesting the possibility to modify strategies against induction of immunological tolerance associated with hybrid vectors. In a study using modified PEI/DNA complex, nanoparticles have suppressed the antigen-specific T-cell responses and lead to regulatory T-cell activation via IFN-αβ-mediated DC activation [70]. However, experimental variations by using different animal strains (C57BL/6 and BALB/c) were also affecting clearance of nanoparticles in mice strains [71]. These reports suggest that further extensive studies are needed to determine the fate of nanoparticles during in vivo administration of vectors. Moreover, the targeting ability of hybrid vectors needs further improvement for efficient gene delivery. The major drawback of viral vectors is their ability to induce oncogenicity and lack of gene transfer specificity [72, 73]. Among other viral vectors, lentiviral vectors can integrate the foreign gene into the host genome and activates proto-oncogenes [74]. Thus, there is a need for further systematic studies for hybrid vectors (viral vector and nanomaterial) to overcome the barriers of individual vectors which hinder their use in the clinical applications.

4.8 Conclusions

Till now, viral and non-viral vectors have been extensively used to deliver a gene of interest to multiple target tissues. Combining both the vectors, hybrid vectors offers immense potential to deliver more than one transgene with tissue specificity. These vectors impart shielding of viral epitope surface to evade host immune response and provide a platform for conjugation of receptor-specific ligands on the surface to enhance targeting ability. However, the development of hybrid vector systems needs exhaustive knowledge of virus structure and the effect of nanomaterial coating on physicochemical properties of vectors. Further, most of the synthetic nanomaterials are also immunogenic in nature which cannot be overlooked. Thus, to generate hybrid system and for other biomedical applications, it is necessary to alleviate the immunogenicity of the synthetic nanomaterial. It is essential to select an appropriate non-immunogenic nanomaterial to develop hybrid vector systems and achieve higher efficacy with minimal side effects.

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