Nanoparticle Coated Viral Vectors for Gene Therapy

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Abstract: *Background*: Recent advances in nanotechnology and gene therapy have created new avenues for therapeutics. However, only a few studies have combined these successful systems for biomedical applications. This review presents an overview of currently available nanoparticle-vector hybrid delivery strategies, the challenges and potential solutions to their widespread use.

Methods: A comprehesive analysis of literarure on the subject was carried out to identify viral vectors that have been coupled with nanomaterials. The outocome of various studies have been depicted with key aspects on their structure and functionality illustrated.

Results: Gene delivery strategies using viral vectors or nanoparticles have been used extensively to deliver functional genes to many target issues. The hybrid vector systems offer immense potential in terms of their abilities to deliver more than one transgene, evade host immune response by potential masking of the immunogenic epitopes on the viral vectors and a sustained release mechanism in the target tissue. However, it is also imperative to understand that the development of such hybrid systems requires extensive knowledge of virus structure and the ability to understand the effect of nanoparticle coating on the physio-chemical properties of the vectors.

Conclusion: Combination of viral and nanoparticle delivery vehicles will require an optimal ratio of nanomaterial with vector to preserve their individual characteristics and still achieve optimal tissue targeting and gene delivery. In addition, the long-term survival of such hybrid systems in the host depends on a rapid yet sustained release of their cargo and avoidance of host immune surveillance.

Keywords: Gene therapy, nanoparticle, vector, immune response, hybrid delivery, gene expression.

INTRODUCTION

Nanotechnology is an exciting medium that combines the physiochemical and biological properties of metallic and non-metallic molecules towards wide-ranging applications spanning imaging and diagnostics to therapeutics. Since the term nanotechnology was coined in 1960s, various modifications to their design have resulted in novel variants with diverse properties [1, 2]. By definition, nanoparticles are in size range of 5-100nm and possess sufficient surface area for binding bi-specific conjugate molecules and/or specific target peptides [3]. Based on its composition, nanoparticles can be categorized as: Polymers [chitosan, dendrimers, latex], Q dots, nanoemulsions, liposomes, carbon-based tubes, metallic [Iron oxide, gold and silver nanoparticles] and ceramic [silica] particles [4]. Nanoparticles possess specific optical, magnetic, chemical and structural properties that impart them with a potential to cross tissue barriers, un-coat and deliver their cargo inside the cells [5-7]. The most common use of nanoparticle is as a carrier with a core containing the 'target specific reagent' which bypasses side-effects associated with pharmaceutical products like antibiotics or chemotherapeutic agents [8]. Due to this advantage, nanoparticles can be used for both vaccination and therapeutic strategies to elicit an immune response or for gene delivery respectively [9]. They can be potentially used for treatment of leukemia, solid tumors and bone disorders.

Replacement gene therapy or delivery of a normal copy of genes through viruses is another promising approach in which recombinant vectors derived from Adenovirus, Retro/Lenti-virus and Adeno-Associated Virus (AAV) have been used for treatment of various genetic disorders [10]. Several clinical gene therapy trials involving viral vectors have failed to reach desired therapeutic end-points [11, 12]. This is primarily because high and persistent levels of transgene expression cannot be sustained due to host immunity. The main objective of either drug or gene delivery is to achieve specificity and stability of the molecules administered into a host [13]. Since host immune response is a common by-product due to viral vector or nanoparticle usage, it is very encouraging that combinations of these two systems have been used recently to optimize therapeutic delivery. For example, nanomaterial coated viral vectors have been proposed to rescue gene therapy vectors from some of the adverse immune events [14, 15]. Taking cue from these examples, this review summarizes the data available on nanoparticle-vector hybrid delivery systems,

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their potential advantages and strategies to enhance the utility of this hybrid system.

GENE THERAPY

Replacement gene therapy is a 'technique to correct genetic defect by transfer of functional gene copies into host cells'. The general divide between two modes of gene transfer, i.e. somatic and germ-line therapy lies in the vertical transmission of genetic traits from parent to progeny. Most gene therapy programs focus on somatic gene delivery. The initial gene delivery events date back to 1990s when the potential of retrovirus for functional reconstitution of adenosine deaminase was established [16]. Currently >1800 clinical trials using different viral vectors are being conducted in different parts of world [10]. Alternative approaches of knocking out the mutated gene (suicide gene therapy) or editing faulty genes (nuclease mediated gene editing) using viral vectors are also gaining popularity [17]. These delivery systems utilize two distinct modes- *ex vivo*, where transduction of DNA or viral vector with gene of interest in recipient cells (e.g. hematopoietic cells) is followed by introduction of these transduced cells into host body or *in vivo* where the vector is administered directly into host by different routes of administration [18]. Each approach has its own merits/demerits and is chosen based on both the disease to be treated and the target tissue for gene transfer. An ideal vector should demonstrate considerable tissue specificity, sustained transgene expression and reduced immunogenicity. Viral vectors have been used with fair amount of success for different genetic disorders like Cystic Fibrosis [19], Hemophilia [20], Leber's Congenital Amaurosis [21] and Severe Combined Immuno Deficiency (SCID) [22] [23]. Three different viruses i.e. adenovirus, retrovirus/lentivirus and adeno-associated virus (AAV) are currently in use as common vehicles for carrying the gene of interest [10]. These vectors have produced variable degrees of success and therefore warrant further modifications for improving the outcome.

HYBRID VIRAL-NANOPARTICLE VECTORS

Advances in developing both viral and non-viral vectors (synthetic vectors) have moved in parallel since early 1980s (Fig. **1**) to overcome the limitations associated with both the vector systems for making gene therapy a more viable option in clinics. To take advantages from vector systems and hybrid systems, a combination of these vectors were developed to achieve efficacy over either of the systems alone. Viral vectors, i.e. Adeno, Retro/Lenti-virus and AAV have been used in conjunction with synthetic materials such as liposomes, dendrimers and hydrogels (Fig. **2**) and have demonstrated immense potential in gene delivery (Table **1**). Adenovirus is an excellent candidate for hybrid vector generation due to its therapeutic efficacy [24-26] in targeting tumor tissue [27, 28]. Adenovirus is known to function effectively with different nanomaterials, for example, with alginate beads [29], chitosan [30, 31] or chitosan-PEG-folate complex [32], PEI [33, 34] etc. Techniques for adding moieties like arginine graft [35], RGD conjugation [36], herceptin [37] or adenovirus surface charge modification [38] have helped in precise vector targeting. These reports serve as excellent templates for the development of nanomaterial coated vector using non-pathogenic viruses like AAV.

DENDRIMER COATED VIRUS PARTICLES

A recent study has analyzed the effect of poly(amido-amine) dendrimer generation 5 (PAMAM-G5) coating on adenovirus mediated gene transfer in a liver cancer xenograft model (Fig. **2A**) [39]. In this study the authors have utilized coated adenovirus particles (Ad5-CMV/NIS) containing the hNIS transgene (sodium iodide symporter) to test their transduction potential and tissue tropism by radioactive iodine isotope (^{123}I) scintigraphy. The results were found to be promising with significant decrease in antibody-mediated neutralization and increase in CAR negative cell adenovirus infection *in vitro*. Further promising results were observed *in*

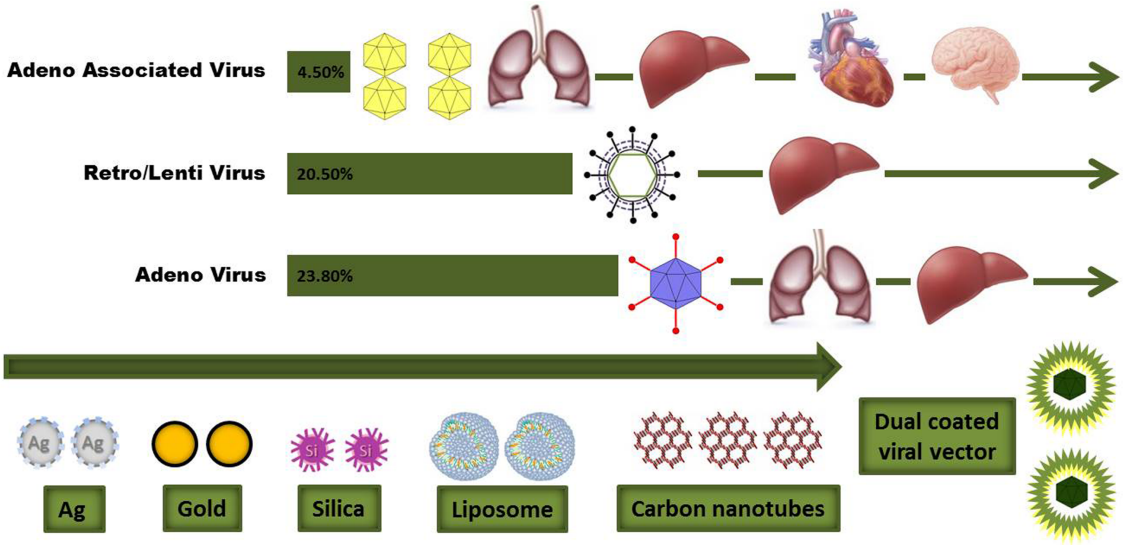

Fig. (1). Scale of nanoparticle and gene therapy vector development. The advances in drug targeting strategies have been depicted to highlight simultaneous progress in generation of nanomaterials (metallic and non-metallic) and viral vector systems e.g. Adenovirus, Adeno-associated virus and Lentivirus.

Table 1. List of nanomaterial coated hybrid viral vectors that have been tested either *in vitro* or *in vivo*.

Viral Vector	Nanomaterial Coating	Transgene	Assay Endpoint	Reference
Adenovirus	Poly(amido-amine) Dendrimer Generation 5 (PAMAM5)	Sodium Iodide Symporter	^{123}I Scintigraphy+ (Radiovirotherapy)	[39]
Lentivirus	Fibrin Hydrogel (+/- Hydroxyl apatite)	Luciferase	Bioluminescence	[46]
Lentivirus	Collagen Hydrogel (+/- Hydroxyl apatite)	Luciferase	Bioluminescence	[45]
AAV	Glyceraldehyde Tag	GFP	*In vitro* transduction	[80]
AAV	Elastin Like Polypeptide	GFP	*In vitro* transduction	[47]
AAV	Elastin Like Polypeptide + poly (ε-Caprolactone)	GFP	*In vitro* transduction	[81]
AAV	Heparin Coated Super Para Magnetic Iron Oxide	GFP	*In vitro* transduction	[48]
AAV	Poly Ethylene Glycol (PEG)	β-Gal	*In vitro* transduction/ serum neutralization	[82]

Table **1** outlines reports of nanomaterial coated viral vectors with specific transgenes. Detection endpoints are mentioned to emphasize ease of utilization with dual vector systems..

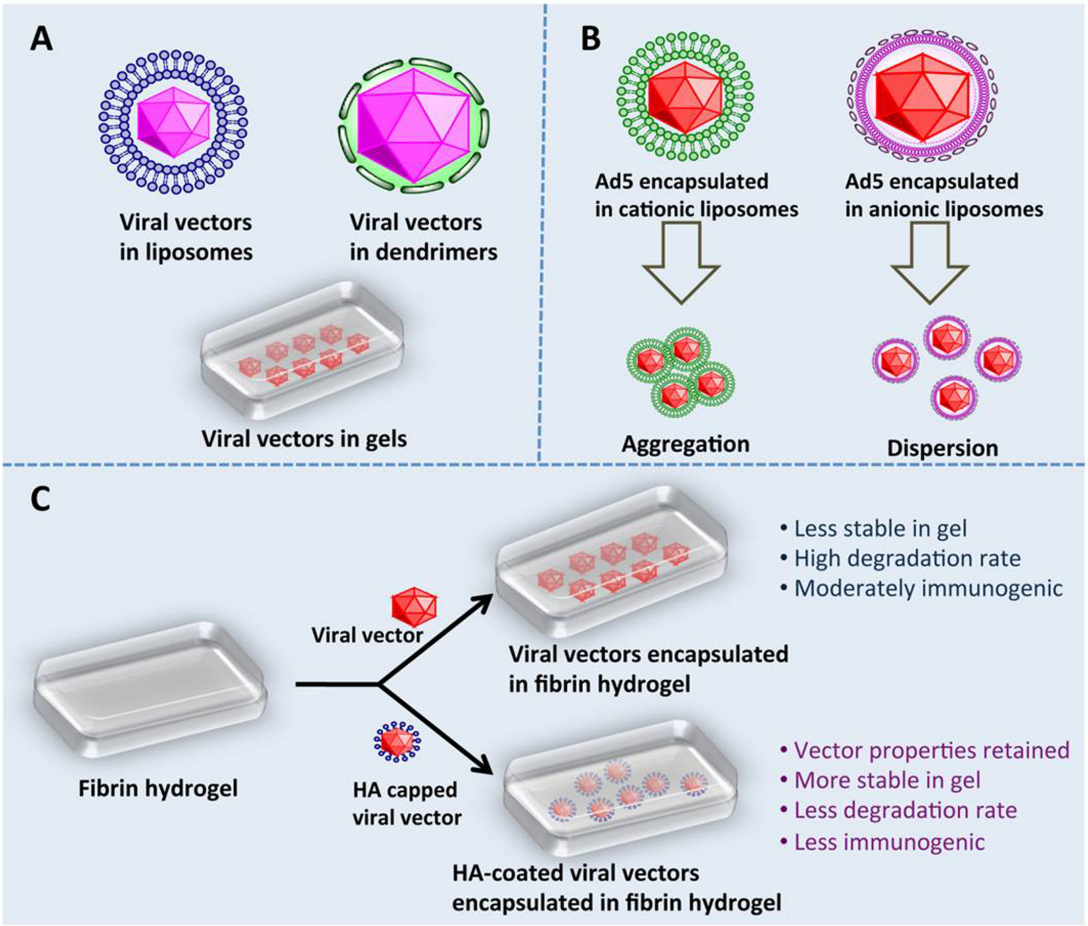

Fig. (2). Hybrid viral nanoparticles. The combination of gene delivery vehicles and nanoparticles offer many advantages including delivery of multiple payloads, escape from host immune system and an ability to achieve enhanced permissibility in specific tissues. This schematic demonstrates the utility of these hybrid systems and depicts, **A**) Types of hybrid viral nanoparticles **B**) Variations between the cationic liposomally bound viral particles and anionic liposomally bound nanoparticles **C**) Fibrin hydrogels with viral particles. Difference between naked viral particles loaded in fibrin hydrogels and hydroxyapatite (HA) coated viral particles loaded fibrin hydrogels are highlighted.

vivo, where such a delivery mechanism resulted in sustained transgene expression and reduction of tumor load in treated mice. These data indicate the high therapeutic potential of the adenovirus hybrid vectors.

LIPOSOMALLY BOUND VIRUS PARTICLES

Viral gene therapy with oncolytic replication selective viruses (OVs) holds great promise for treating cancer as they specifically replicate within cancer cells and induce

apoptosis [40]. Human clinical trials in patients with advanced stages of cancer have shown significant positive responses and an increase in OV based gene therapy [41]. However, rapid clearance by the reticuloendothelial (RE) system in the liver and neutralization by antibodies limit their distribution into the tumour cells which in turn affect their efficacy [42]. To address this limitation, Yotnda P. *et al*. have encapsulated adenoviral vectors in bilamellar cationic liposomes, composed of (1,2-dioleoyloxypropyl)-*N*,*N*,*N*-trimethylammonium chloride (DOTAP) and cholesterol [43] (Fig. **2B**). They demonstrated that liposomally bound adenovirus could effectively transfect the target cells that lack adenoviral receptors or in which the recipient already has or develops a neutralizing antibody response, when compared with naked adenovirus particles. Despite the promising *in vitro* results, their clinical applications have been hindered due to systemic toxicity, low tissue specificity, and poor serum stability. To address these concerns Mendez N. *et al*. encapsulated adenoviral vectors in anionic bilamellar liposomes using a nontoxic material, refined lecithin, a mixture of phosphatidylcholine, phosphatidylethanolamine, inositol phosphatides, and other phospholipids as well as cholesterol and polyethylene glycol-2000 (PEG2000) to encapsulate adenovirus5 (Ad5) (Fig. **2B**) [44]. Their findings convincingly demonstrated that liposomally encapsulated adenoviral vectors showed superior transfection properties in cancer cells than the naked Ad5 and also could be used for repeated administrations *in vivo*. More importantly, stability of the anionic liposomal virus particles significantly increased and showed monodispersion even after 32 hours, whereas, cationic liposomal virus particles aggregated after an hour (Fig. **2B**). These findings hold promise for their clinical applications [44].

VIRUS PARTICLES EMBEDDED IN GELS

In an attempt to generate a better transduction profile with lentiviruses, Shin and Shea have reported the use of collagen hydrogel in combination with hydroxyapatite for encapuslating the lentivirus particles [45]. The effect of nanomaterial encasing as well as hydrogel degradation kinetics on host cell mediated transgene expression was studied both *in vitro* and *in vivo*. An increase in transduction efficency (~80%) was noted for encapsulated lentivirus in invasive C6 glioma cells. In addition, the composition of collagen hydrogel (0.05%, 0.15%, 0.3%) was found to be important for virion release and cell migration from the surrounding tissue. In animal models, the effect of hydroxyapatite containing collagen gel on lentivirus encoded luciferase gene expression in CD-1 male mice was marginal with a 33% increase seen in comparison to only gel encapusulated virus. Similarly, Kidd *et al.* have demonstrated the utility of fibrin hydrogel and hydroxyapatite coated lentivirus particles for localized vector transduction in CD-1 mice (Fig. **2C**) [46]. Importantly, the encapsulation by fibrin hydrogel did not affect virus infectivity or their cellular infiltration.

To generate high performance delivery systems using AAV, vectors have been combined with elastin-like polypeptide (ELP). Subsequently they were tested for their infectivity into murine fibroblasts (NIH3T3) and human neural stem cells (NSCs) [47]. An AAV variant r3.45, obtained by directed evolution was used for this study. The authors have shown a significant difference in efficacy of ELP conjugated AAV r3.45 transduction as compared to control groups, which argues well for their potential use in NSCs for dissection and treatment of various neurodegenerative disorders. AAV vectors encapsulated within 'smart biomaterials', ELP in combination with (PCL) [poly (ε-Caprolactone)] for electro-spinning, have also been used to maximize AAV contact with tissue for efficient and sustained gene transfer in tissue engineering applications [14]. Heparin coated super paramagnetic iron oxide nanoparticle (SPIONs) in combination with AAV variant 3.45 have been reported for enhanced gene delivery in to different cell lines, e.g. HEK293T and PC12 cell lines [48]. A short exposure of <180 minutes was effective in transducing the target cells and equaled the results achieved with conventional 24 hour incubation period for cell transduction. In addition, due to the magnetically enhanced AAV transduction, critical phenotypes such as the nerve growth factor expression and the neurite extension in PC12 cells were improved. Taken together, these data suggest that the combination of vectors and nanoparticles have several benefits over the conventional delivery methods. Nonetheless their widespread use will require that certain challenges related to tissue specificity, host immune response and kinetics of nanoparticle-vector hybrid delivery be studied exhaustively in an *in vivo* setting.

CHALLENGES

The influx of nanoparticles inside the host cells in a large quantity generates a concentration gradient across vascular endothelium which is known to restrict their entry [49]. This also results in aberrant distribution of nanoparticles and in stimulation of the residential monocytic-phagocytic system (Fig. **3**) resulting in compromised therapeutic efficacy. In addition, several properties of hybrid nanoparticles [size, solubility and route of delivery [50], stability, purity and zeta potential] determine their intracellular processing [51] [50]. These multivalent molecules mimic naturally found biomolecules in circulatory system and viruses [52] and are thus processed similarly *in vivo* [53] [4] as described below.

Immune response associated with nanoparticle circulation has been observed in various drug delivery and vaccination studies [6]. The magnitude and duration of the innate response is dependent on tissue targeted (e.g. skin, lungs, gut) each of which differ in number of residential immune cells [54] [55]. After cellular entry, nanoparticle fragments act to incite either innate or adaptive immune response by a cascade of events starting from antigen presentation by APCs to their expulsion by exocytosis or resulting in cellular apoptosis [56]. Endothelial cell injury and malfunction often act as a first sign of their toxic effects on vascular system (Fig. **3**) [57]. Table **2** summarizes the effects of various nanoparticles on the immune system but it must be noted that an overlap between these events is frequently observed.

A summary of possible events leading to adaptive immunity to nanoparticles is detailed in Fig. (**4**). Immature dendritic cells (DC) from nearby draining lymph nodes

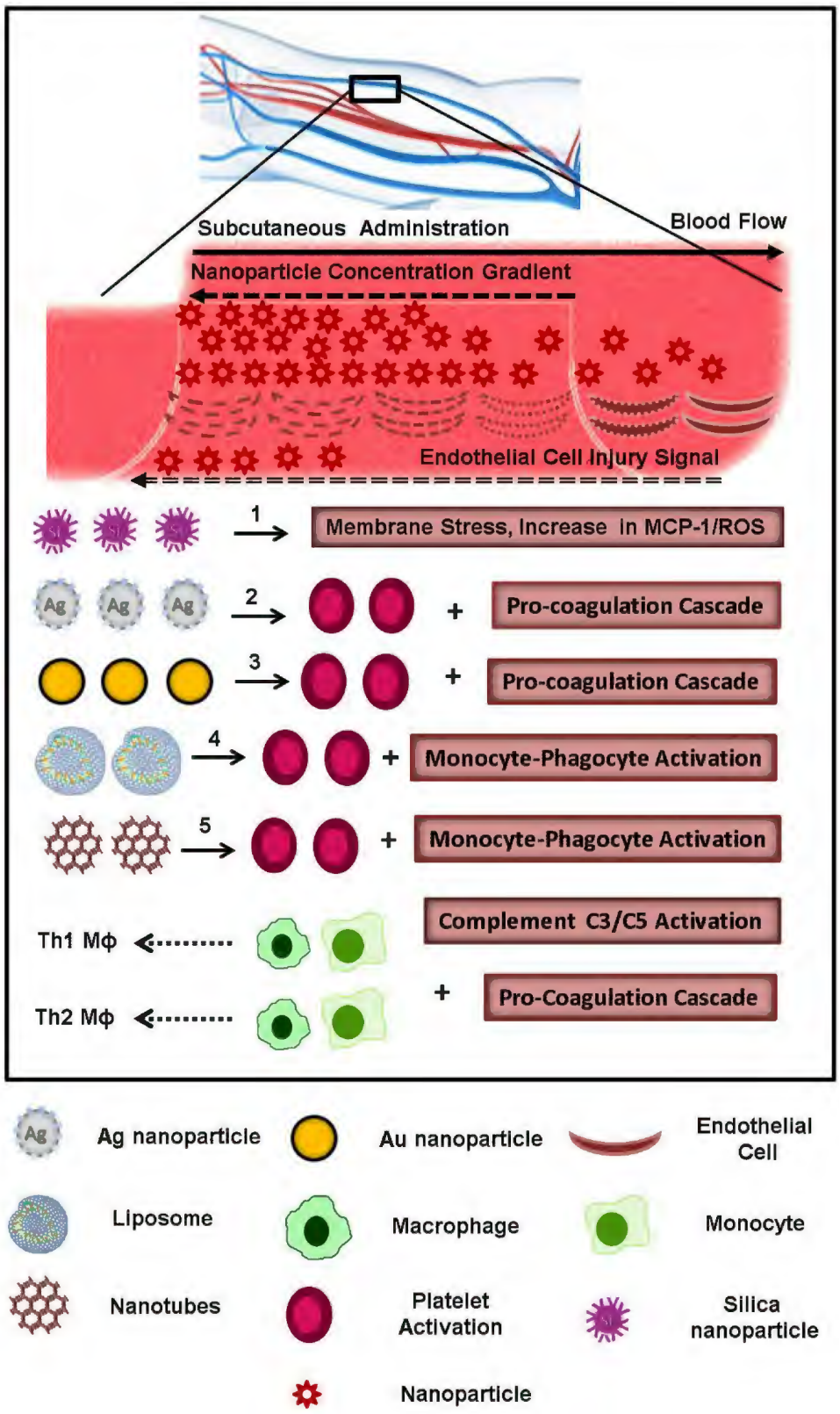

Fig. (3). Innate immune response against nanoparticles. The entry of nanoparticles through endothelial cells is a primary event that triggers a cascade of reactions towards nanoparticles or their fragments [68]. Innate immune response towards them differs substantially and depends on the nanoparticle size, shape, charge and associated ligand/peptide molecules as well as route of entry [69-71]. Endothelial membrane disruption due to nanoparticle infiltration acts as a signal of injury that activates vascular system along the nanoparticle concentration gradient. Events 1-5 depict five different nanoparticles, i.e. silica, silver, gold, liposome and carbon nanotubes and the innate response noted against them. Macrophage mediated phagocytosis of nanoparticle/ fragment involves multiple events including macrophage migration and differentiation in response to chemokines/cytokines that trigger Th1Mφ /Th2Mφ cells [72-74].

capture the nanoparticle antigen and activate T cell differentiation and they also stimulate B cells [58]. Activation and functional antigen presentation by DCs are dependent on the secretion of inflammatory cytokines like TNF-α and IL-1β along with the presence of co-stimulatory receptor ligands CD80 (B7-1) and CD86 (B7-2) [59]. Activated DCs display multiple signals along with processed antigenic peptides in conjunction with MHC class I and II molecules to naïve T cells bearing TCR (T cell antigen receptor) [60]. Co-stimulatory signals CD80/86 from APC interact with T cell receptors like CD28; this is accompanied by secretion of cytokines, e.g. IL-12, IL-4, IL-6, TGF-β which signal the naïve T cells to differentiate into Th1, Th2 or Th17 cells. The antigen presentation is likely to involve the MHC class II loading pathway [61]. This could result in only a limited number of CD8+ T cells generated as the soluble or endocytosed antigens can be presented to only specific groups of DCs present in spleen or lymph nodes [62]. These also suggest that it may be possible to devise strategies for the induction of immunological tolerance against the hybrid vectors. Indeed, modified DNA/PEI complex nanoparticles have been reported to suppress antigen specific T cell responses and result in Regulatory T cell activation through IFN-αβ mediated DC activation [63]. Nonetheless, experimental variations induced by various animal strains (C57BL/6 and BALB/c) are also known to affect nanoparticle clearance in mice strains [64]. Taken together, these data suggest that further extensive studies are needed to assess the immunological fate of the nanoparticles during vector transfer *in vivo*.

The other major potential of these hybrid vectors could be in improving the specificity of gene delivery of viral vectors. The most important concerns of viral vectors are their potential oncogenicity [65] and the lack of gene transfer specificity [66]. In particular, lenti-viral vectors are able to integrate the transgene into the host genome and activate proto-oncogenes [67]. Thus, with further systematic studies with the combination of these two delivery vehicles, nanomaterial and vector, it will be also possible to solve the disadvantage of non-specific gene transfer inherent to viral vectors.

CONCLUSION

Gene delivery strategies using viral vectors or nanoparticles have been used extensively to deliver functional genes to many target issues. The hybrid vector systems offer immense potential in terms of their abilities to deliver more than one transgene, evade host immune response by potential masking of the immunogenic epitopes on the viral vectors and a sustained release mechanism in the target tissue. However, it is also imperative to understand that the development of such hybrid systems requires extensive knowledge of virus structure and the ability to

Table 2. Immune response reported with various different nanoparticle formulations.

Nanoparticle	Primary Event/Effector Molecule/Cellular Component	Reference
Gold nanoparticles	Platelet activation, plasma membrane disruption	[53, 83]
Silver nanoparticles	Cyto-toxic effects on Endothelial cells, pro-inflammatory cytokine, chemokine production, NF-KB pathway activation, free radical generation	[57]
Metallic oxide nanoparticles	Chemokine receptor molecule (Type 4, CXCR4) Adhesion molecule expression levels	[84]
Silica nanoparticles	Nitric oxide generation, Peroxynitrite production Up-regulation of ICAM1, VCAM1, IL-8 and IL-6, NF-KB activation, cell damage Reactive oxygen species generation, apoptotic signal molecules and transcription factors up-regulation, release of Tissue Factor, IL-6, IL-8, MCP-1 and ROS	[85] [86]
Carbon nano-tubes	Complement mediated opsonisation, C3/C5 & Membrane attack complex formation Endothelial membrane leakage Platelet activation and aggregation, degranulation, ATP release Oxidative stress induction, cytokine production (TNF-α), IL-1β and IL-8 Inflammation	[87] [88] [89] [56, 90-93] [94-98]
Dendrimers	Endothelial cyto-toxicity, endotoxin induced pro-coagulant activity	[92, 99, 100]
Liposome	Expression of macrophage maturation marker and polarization of monocyte Inhibition of macrophage migration Endothelial cell cyto-toxicity	[101] [72]
Cationic Lipid (RPR206252)	TNF-α, IL-1β, IL-6, IFN-γ production NF-KB activation, TLR-2 and NLRP3 activation	[102]
Polystyrene latex particle	Platelet activation and aggregation, up-regulation of adhesion receptor	[103]
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	[104]
Per-fluoro carbon emulsion	Complement system activation	[105, 106]

Table **2** summarizes the effects of nano-particle processing and distribution on different immune system components. An overlap between immune response events between different categories of particles is frequently observed.

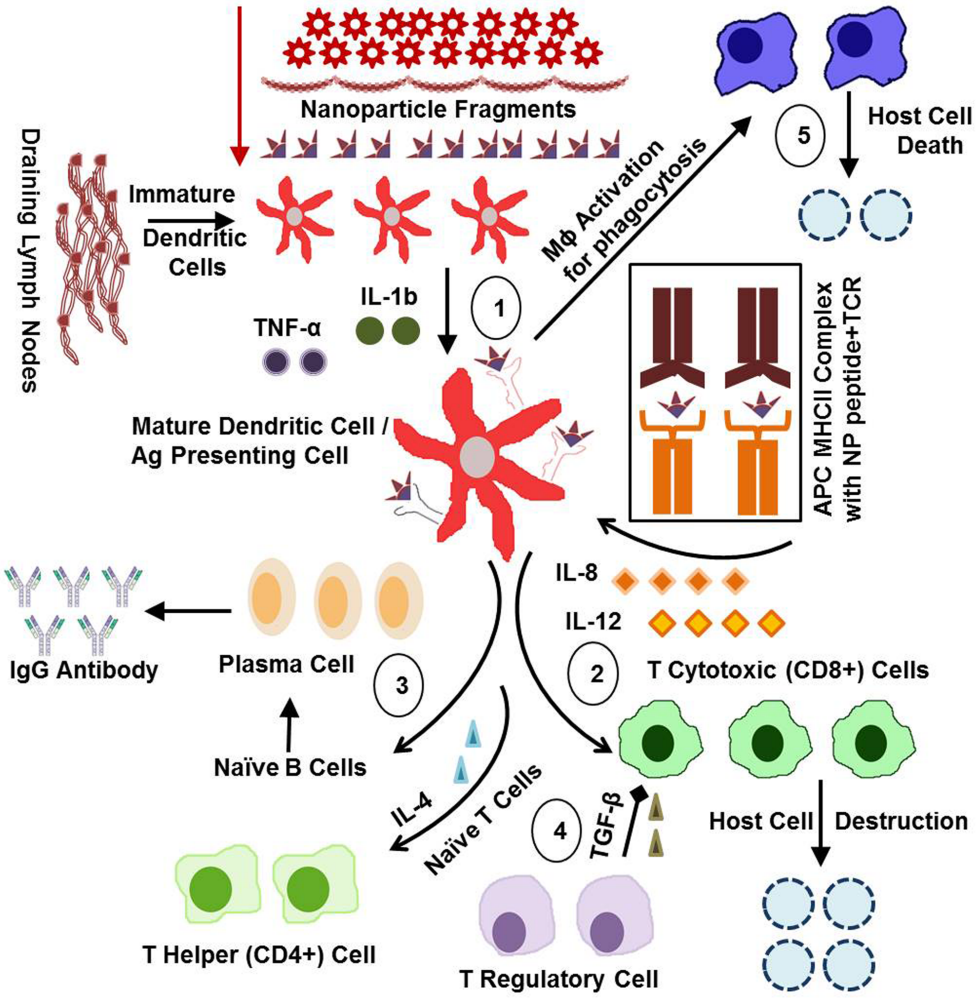

Fig. (4). Adaptive immune response against nanoparticles. This sketch depicts the adaptive immunity noted against the nanoparticles. 1) Dendritic cells act as a link between innate and adaptive immune system and prompt their cross activation through several signals (MHCI/II-peptide complex, CD80-CD80L etc.) [59, 60, 62, 75]. Movement of DCs bearing the peptide/MHC complex towards lymph nodes [76] is a decisive 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 secreted 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 results in host dendritic and cytotoxic T cell population activation [77]. B cell activation and antibody generation has also been reported for nanoparticles coated with peptide ligands. 4) Bypass of T cell or B cell mediated response towards nanoparticles has also been reported by activation of T-regulatory cells and the suppression of pro-inflammatory molecules like IL-2, IL-6, TNF-α etc. 5) Macrophage activation and differentiation by dendritic cells and nanoparticle phagocytosis mark the expulsion of nanoparticle from the host tissue [78, 79].

understand the effect of nanoparticle coating on the physio-chemical properties of the vectors. On a cautious note, the fact that often most of the nanomaterials are immunogenic on their own can-not be overlooked. Mitigating immunogenicity of the synthetic nanomaterials is a critical task to enhance their applications in biomedical field. Thus selecting an appropriate non-immunogenic nanomaterial to generate hybrid system is pivotal in achieving success with this approach. In addition, host immunity to these hybrid particles needs to be comprehensively studied, particularly in higher animal models, prior to their potential use in humans.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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