Self-assembled Viral Nanoparticles as Targeted Anticancer Vehicles

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Abstract Viral nanoparticles (VNPs) comprise a variety of mammalian viruses, plant viruses, and bacteriophages, that have been adopted as building blocks and supramolecular templates in nanotechnology. VNPs demonstrate the dynamic, monodisperse, polyvalent, and symmetrical architectures which represent examples of such biological templates. These programmable scaffolds have been exploited for genetic and chemical manipulation for displaying of targeted moieties together with encapsulation of various payloads for diagnosis or therapeutic intervention. The drug delivery system based on VNPs offer diverse advantages over synthetic nanoparticles, including biocompatibility, biodegradability, water solubility, and high uptake capability. Here we summarize the recent progress of VNPs especially as targeted anticancer vehicles from the encapsulation and surface modification mechanisms, involved viruses and VNPs, to their application potentials.

Keywords: viral nanoparticles (VNPs), self-assembly, anticancer, drug delivery, nanotechnology

1. Introduction

Recent advances in nanotechnology have facilitated the development of medical science, especially in the field of drug delivery for therapeutic and/or imaging applications [1]. Numerous nanomaterials have been investigated for their potentials as drug carriers, including liposomes, polymers, dendrimers, inorganic nanoparticles, and protein-based nanoparticles [2-4]. Nanoparticles (NPs) for drug delivery systems are capable of carrying large quantities of therapeutics, targeting particular cells of interest, and presenting sufficient physiochemical properties [5,6]. However, some of these synthetic carriers are limited by their biocompatibility, toxicity, and/or low efficiency for clinical utilization [7].

Viral nanoparticles (VNPs) are virus-derived formulations of protein-polynucleotides hybridized supramolecular assemblies that can be adopted as building blocks and templates due to their sophisticated architectures [8]. The essential nature of viruses is to infect a host cell, replicate, package its nucleic acid, and exit the cell, during which they display the exceptional plasticity of dynamic, self-assembled systems with precise dimensions and monodisperse structures [9]. VNPs include bacteriophages, plant and animal viruses, with a distinctive diversity of shapes (icosahedral, helical, enveloped, or complex) and a variety of sizes (from tens to hundreds of nanometers) [10]. Virus-like particles (VLPs) are a subset of VNPs consisted of multiple protein subunits without genomic materials that makes them noninfectious and unable to replicate [11]. VLPs and VNPs can be produced on large scale in short time either from natural infected tissues or heterologous expression systems. These protein-based nanoparticles possess numerous advantages over other synthetic NPs, such as water solubility, immune evasion, biocompatibility, and bioavailability [12,13].

VNPs present “programmable” scaffolds because the viral protein cages can be modified both chemically and genetically to load therapeutic cargoes and install targeting ligands [14,15]. Chemical modification and genetic manipulation on the outer surfaces and inner cavities of VNPs have been employed for the construction of multivalent...
and multifunctional viral capsid-based materials [16,17]. VNPs are remarkably stable and robust to endure the pressure and degradation in the cellular environment, meanwhile they are sensitive to recognize signals and release payloads when instructed [18]. This flexibility confers them suitable tools for material science and pharmaceutics research.

Various VNPs have been extensively studied for targeted delivery and imaging potentials due to their capability of transfection, e.g. cowpea mosaic virus (CPMV), cowpea chlorotic mottle virus (CCMV), tobacco mosaic virus (TMV), and bacteriophages such as MS2, P22, and Qβ [19-23]. Viral vectors derived from adenovirus (Ad), adeno-associated virus (AAV), herpes simplex virus (HSV), and other oncolytic viruses have also been widely used as gene delivery vehicles and even clinically approved vaccines [24-26]. In this review, we focus on the recent progress of VNPs as targeted anticancer vehicles including their encapsulation and surface modification mechanisms, the involved viruses and VNPs, and their application potentials.

2. Encapsulation and Surface Modification Mechanisms by VNPs

VNPs are flexible and amenable for packaging and delivering a broad range of cargoes such as nucleic acids, proteins, polymers, and chemotherapeutic drugs [27,28]. The interior interface of VNPs can be used to encapsulate, diffuse, absorb, or conjugate functional payloads and the exterior surface can also be decorated by multiple targeting moieties [29].

2.1. Encapsulation by VNPs

As shown in Fig. 1, different strategies such as self-assembly, diffusion, chemical conjugation, and genetic manipulation have been explored for loading cargoes inside the viral capsids. The genomic materials are generally packaged within the capsids by a reversible disassembly and reassembly process in vivo, and the principle regulating this process can be utilized for loading other cargoes [30]. Electrostatic interaction serves as the thermodynamic force driving efficient packaging and assembly of negatively charged molecules inside the positively charged interior surfaces of the capsids [31]. Bancroft et al. first revealed the encapsulation of polyanions instead of viral RNA by CCMV capsids, which was triggered by mixing the capsid protein subunits with functional cargoes at specific buffer conditions, ionic strength, temperature, and pH ranges in vitro [32,33]. As a model plant virus, CCMV has been widely studied by researchers for the capability to encapsulate various materials [34-36]. CCMV capsids can disassemble into protein dimers and release loaded cargoes at physiological pH or high ionic strength (I ~ 1 M), and reassemble as the capsids when adjusting the buffer pH between 3 and 6 under low ionic strength (I ~ 0.1 M) [37]. Taking advantage of this process, anionic polyoxometalate, negatively charged polyelectrolytes, and silencing RNA (siRNA) have been successfully encapsulated into CCMV capsids with potential pharmaceutical applications [38,39]. Interestingly, CCMV capsids expand into a “swollen state” with 10% radially larger and 60 open pores of ca. 2 nm on the cage at pH 5–6.5 and I ~ 0.1 M [40]. Small molecules can diffuse freely into and out of the cavity with conditions changed, that provides a particular molecular

Fig. 1. Schematic diagram of the encapsulation mechanisms by viral nanoparticles to load cargoes. (A) Self-assembly around cargoes driven by electrostatic interaction under different pH and buffer conditions. (B) Diffusion of cargoes within viral capsids through open pores or channels due to altering pH and salt concentrations. (C) Chemical conjugation between the amino acid residues of capsid protein subunit and cargo moieties. (D) Genetic manipulation to introduce biospecific sequences for covalent attachment with cargo moieties.
gating mechanism for the encapsulation and release of functional cargoes [41]. Similar open channels of 11–13 Å-diameter are also formed by red clover necrotic mosaic virus (RCNMV) capsids with the removal of calcium and magnesium ions from the buffer, and the supplementation of these ions closes the formed channels [19]. By passive diffusion through these channels, doxorubicin (DOX) was loaded inside RCNMV capsids that were conjugated with a CD46-targeting peptide, which then showed significant cytotoxicity to HeLa cells in vitro [42].

In addition, chemical conjugation can be employed to encapsidate cargo molecules inside the capsids by covalent bond between the carboxylate groups of amino acid residues and the amine groups of cargo molecules [43]. The well-defined viral cavity offers the circumstance for cargo loading and synthesis inside the capsids [44]. For instance, the encapsidation of DOX by the major structural protein VP6 of rotavirus was accomplished through the amide bond between the carboxyl group of VP6 and the amine group of DOX [45]. The targeting moiety of lactobionic acid for hepatocytes or hepatoma cells bearing asialoglycoprotein receptors (ASGPRs) was then conjugated onto DOX-loaded VLPs, which exhibited immunofluorescence in human hepatocellular carcinomas (HepG2) cells in vitro. Building of similar strategy, cytosine deaminase and purine-nucleoside phosphorylase, two prodrug-converting enzymes, were tagged with a positively charged Rev peptide and encapsidated by bacteriophage Qβ fused with a bifunctional linker RNA (biRNA) that recruits Rev-tagged cargo via noncovalent attachment of biRNA with capsid protein and Rev peptide [46].

Genetic manipulation provides another approach for the directed loading of cargoes by VNPs [47]. The cargo and capsid protein can be genetically manipulated separately or simultaneously to introduce biospecific sequences for covalent attachment with each other [48]. CCMV capsid protein and enhanced green fluorescent protein (EGFP) were fusion-expressed with the heterodimeric coiled-coil linkers of K-coil (KIAALKE)$_3$ and E-coil (EIAALEK)$_3$, respectively [49]. The in vitro assembly of EGFP-packaged capsids were achieved by specific heterodimerization between K-coil and E-coil. Making use of biotin/streptavidin affinity, the scaffolding proteins of bacteriophage P22 procapsids were genetically modified with streptavidin and biotin-4-fluorescein was then encapsulated via biotin/streptavidin linker [50].

2.2. Surface modification of VNPs
Besides the strategies of encapsulation, VNPs can also integrate cargo molecules on the exterior surface of the capsids through bioconjugation with exposed amino acid residues (Fig. 2) [51]. The amino acid residues have been

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Fig. 2. Schematic diagram of the conjugation mechanisms on the exterior surface of the capsids of VNPs through bioconjugation to load cargoes. (A) NHS (N-hydroxysuccinimide) esters acylation. (B) Copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. (C) Hydrazine ligation.
illustrated to be applicable and addressable by click chemistries, including endogenous amino acids such as lysines, glutamic or aspartic acids, and tyrosines, and genetically introduced ones such as cysteines [52,53].

NHS (N-hydroxysuccinimide) esters acylation is the simplest and most common technique used for surface modification of exposed amines, which involves carbodiimide crosslinking of carboxylates (-COOH) with primary amines (-NH₂) [54]. Wang et al. first demonstrated NHS-esters of fluorescein or biotin to conjugate with CPMV, and the obtaining VLPs showed a similar structure with self-assembled dendrimers or metallic NPs [55]. Varieties of reactive residues including tyrosines, carboxylate groups, natural and genetically introduced cysteines and histidine tags were decorated with small fluorescence molecules and peptides [56].

Copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction has resulted in numerous applications in chemoselective ligation on virus surfaces since its first report in 2002 [57]. The azide and alkyne moiety can be attached to the desired cargo molecules and lysine, cysteine, or tyrosine residues on the surface of VNPs, respectively [58]. The selective reactivity between azide and alkyne groups makes CuAAC reaction more efficient with a notably lower molar excess of substrates compared with NHS-ester based chemistry [59]. CPMV was first exploited for the grafting of small fluorescent molecules via CuAAC reaction [60]. A mutant CPMV conjugated with the folic acid- polyethylene glycol (PEG) ligand showed specific recognition and binding with the folate receptor of tumour cells while complete elimination with normal cells [61]. Our group also employed CuAAC reaction for the attachment of oligo-ethylene glycol short chain and Arg-Gly-Asp (RGD) peptide on the exterior surface of CCMV, resulting in opposite adhesion behaviors with HeLa cells [62].

Hydrazone ligation provides another chemoselective strategy for bioconjugation of VNPs with ligands under relatively less stable conditions. Benzaldehyde is first introduced to VNPs and then reacted with cargo molecules equipped with a hydrazido group via hydrazone ligation [63]. CPMV was assembled with individually tunable levels of vascular endothelial growth factor receptor (VEGFR) 1 ligand and fluorescent PEGylated peptide using this approach, which recognized VEGFR1 both in endothelial cell lines and tumour xenografted mice [64].

Although NHS esters acylation is simple, the yield and efficiency is not high compared to other bioconjugation reactions. CuAAC reaction is therefore preferred for its bioorthogonal, quicker and more readily proceeds than NHS or maleimide reactions. Hydrazone ligation is chemoselective and shows an absorbance near 350 nm, which can be utilized VNPs conjugation when not stable in click chemistry reaction.

3. Viral Nanoparticles for Cancer Therapy

As illustrated in Fig. 3, a variety of viruses and VNPs exhibit the ability to carry the active drugs to cancer cells utilizing the unique pathophysiology of tumors, such as their enhanced permeability and retention effect and the tumor microenvironment. Multiple targeting molecules

![Fig. 3. Schematic diagram demonstrating the principle of viral nanoparticles as targeted anticancer vehicles for the treatment of cancer. (A) Viral nanoparticles encapsulated with anticancer therapeutics (or imaging agents) specifically target and bind cell membrane of cancer cells with overexpression of receptors. (B) Viral nanoparticles do not interfere with normal cells without overexpression of receptors.](image-url)
including tumor-homing peptides and targeting ligands or antibodies have been displayed on VNP by chemical or genetic manners for anticancer drug delivery and tissue targeting [65]. The tumor-homing peptides involve Asp-Gly-Arg (NGR), RGD and IRGD motif, human epidermal receptor (HER), 31-amino acid fragment of human high mobility group protein 2 (F3), epidermal growth factor receptor (EGFR), and VEGFR, while targeting ligands or antibodies include transferrin, folic acid (FA), and single-chain antibodies [66,67]. In addition to the artificial targeting, a subset of viruses, e.g. oncolytic viruses, present natural affinity for receptors which are usually up-regulated on tumor cells [68].

3.1. Mammalian virus nanoparticles

Mammalian viruses composed of viral envelope components or inactivated viral particles have been developed as delivery vehicles for therapeutic molecules by many researchers [69,70]. These virosomes demonstrate selective targeting and replication in tumor cells, meanwhile they have disadvantages such as immunogenicity and instability in the circulation. Among them, oncolytic viruses (OVs) are a group of viruses that selectively propagate and destroy cancer cells without causing excessive damage to normal tissues [71].

Adenovirus (Ad) is a non-enveloped dsDNA virus and has been the most extensively utilized OV model for human gene therapy [72]. Genetic manipulations of Ad NPs have been widely used for the improvement of performance and pathogenicity. For instance, Ad DNX-2401 (Delta-24-RGD) was constructed by a 24-bp deletion in early 1 A adenoviral gene (E1A) which caused the virus incapable to replicate in normal cells while replication capable in tumor cells, and an RGD-motif was grafted in the fiber H-loop which led the virus to bind with α5β1 or αvβ5 integrins and enhance targeting to tumor cells [73]. Further treatment with DNX-2401 in phase I trial showed significant responses to long-term survival in recurrent malignant gliomas by prolonging survival in 20% of patients [74]. The hexon of adenovirus type 5 (Ad5) was shielded by humanized single-chain antibody variable fragment (scFv) to reduce off-targeting type 5 (Ad5) was shielded by humanized single-chain antibodies [66,67]. In addition to the artificial targeting, a subset of viruses, e.g. oncolytic viruses, present natural affinity for receptors which are usually up-regulated on tumor cells [68].

HSV-1 (Edmonston vaccine strains of MV) and measles virus (MV) have also been explored for cancer treatment [82,83]. Edmonston vaccine strains of MV exhibit significant antitumor activity against glioma stem cells in vitro and in vivo, and the recombinant MV-NIS encoding the human thyroidal sodium-iodide symporter (NIS) resulted in effective concentration of radioactive iodine in human tumor cells which can be used for gamma-camera imaging of iodine-123 (123I) uptake and radiotherapy by 131I administration [84]. Phase I trial of patients with recurrent or refractory multiple myeloma using MV-NIS showed relative safety and suggested to be a novel approach for the targeted infection and destruction of disseminated myeloma [85].

Paramyxovirus such as Newcastle disease virus (NDV) and measles virus (MV) have also been explored for cancer treatment [82,83]. Edmonston vaccine strains of MV exhibit significant antitumor activity against glioma stem cells in vitro and in vivo, and the recombinant MV-NIS encoding the human thyroidal sodium-iodide symporter (NIS) resulted in effective concentration of radioactive iodine in human tumor cells which can be used for gamma-camera imaging of iodine-123 (123I) uptake and radiotherapy by 131I administration [84]. Phase I trial of patients with recurrent or refractory multiple myeloma using MV-NIS showed relative safety and suggested to be a novel approach for the targeted infection and destruction of disseminated myeloma [85].

The truncated hepatitis B virus core antigen (tHBcAg) was engineered to construct pH-responsive VNP for controlled drug delivery [86]. The tHBcAg VNP were loaded with DOX and polyacrylic acid (PAA), and then FA was conjugated to the external surface. The resulting FA-tHBcAg-PAA-DOX nanoparticles were pH-responsive and released free DOX under simulated physiological conditions, leading to increased uptake of DOX in colorectal cancer cells and enhanced antitumour effects. The envelope protein of Moloney murine leukemia virus (Mo-MLV) was used to encapsulate fluorescent NPs and covalently linked with β-lactamase (βlac) [87]. The coated Mo-NPs mimicked native virus by binding mCAT-1 receptor and βlac activity was only detected in the cytosol of tumor cells indicating Mo-NPs could escape endosomes and then locate in the cytoplasm.

To date, only two genetically engineered OVs (Oncorine
and T-VEC) have been approved as commercially available drugs. However, the major limitation of OVs and mammalian viruses is their immunogenicity and the humoral response, whether pre-existing or elicited by virus administration, which neutralizes their infectivity and limits the therapeutic effects [88].

3.2. Plant virus nanoparticles

Plant virus nanoparticles present an alternative approach for drug delivery vehicles. VNPs derived from plant viruses and bacteriophages are especially advantageous as they are less pathogenic and virulent in humans compared with mammalian viruses [9,10]. Plant viruses such as CPMV, CCMV, TMV, and potato virus X (PVX) demonstrate broad tissue distribution and rapid clearance with good blood and tissue biocompatibility [89,90]. Although there is no clinically approved nanomedicine based on plant viruses or bacteriophages, several preclinical trials are undergoing toward entering translational development.

CPMV has been explored as model plant virus for biomedical imaging and diseases targeting [91]. Apart from the aforementioned surface modification of CPMV by different ligand or peptides, the interior cavity of CPMV was also loaded with antineoplastic chemotherapeutics. CPMV was utilized to deliver mitoxantrone (MTO), a topoisomerase II inhibitor against primary brain tumor and glioblastoma multiforme (GBM), and the obtained MTO-CPMV demonstrated enhanced cytotoxic effects against GBM cells in vitro [92]. HER2 epitope was attached with CPMV for epitope delivery and CPMV-HER2 inhibited the progression and metastasis of DDHER2 primary tumors in Balb/c mice as well as prolonged the survival of mice [93].

CCMV has also been widely exploited for nanoscale fabrication and drug delivery [94]. A programmable chimeric CCMV was constructed for targeted drug delivery through the assembly of CCMV capsid protein conjugated with various molecules, i.e. CFSE/Alexa fluor as imaging agent, FA as targeting agent, and DOX as anticancer drug [95]. The chimeric system showed highly specific targeting to folate receptor and approximately 300% more cytotoxicity against Michigan cancer foundation-7 (MCF7) cells. Our group exploited the fusion expression of CCMV capsid protein with tumor-homing peptide F3, the resulting F3-CCMV encapsulated near-infrared (NIR) fluorescent dye IR780 iodide for targeting delivery into MCF7 cells and induced photothermal effect in vitro [96].

TMV provides a fascinating platform for efficient drug delivery because of their structure transition between stacks of disks and rod-like spirals [97]. The platinum-based drug candidates phenanthriplatin (PhenPt) and cisplatin were integrated into the TMV cavity via either charge-driven reaction or covalent attachment [98,99]. PhenPt-TMV exhibited matched delivery efficacy in breast cancer MDA-MB-231 xenografted mouse model compared with free PhenPt [98]. Cisplatin-loaded TMV showed efficient uptake by ovarian cancer cells and superior cytotoxicity and DNA double strand breakage in both platinum-sensitive and resistant cancer cells when compared to free cisplatin [99]. Another filamentous plant virus PVX was decorated with EGF-targeting peptide ligand GE11 (YHWYGYPQNV1), which presented successful targeting and partitioning toward EGFR+ cancer cells and macrophages [100]. PVX was also employed to carry Herceptin (Trastuzumab) for targeted therapy in HER2+ breast cancer, inducing a much higher cell apoptosis in HER2+ cells compared with free Herceptin at similar concentration [101].

3.3. Bacteriophages

Bacteriophage MS2 possess diverse properties suitable for targeted drug delivery and cell imaging. MS2 capsids equipped with cell binding peptide SP94 (FSIIIHTPILPL) exhibited 104-fold higher affinity for human hepatocellular carcinoma (HCC) cells than other non-specific cells [102]. SP94-MS2 was further loaded with DOX, cisplatin, and 5-fluorouracil showing selective killing of HCC cells, while SP94-MS2 encapsidated a siRNA cocktail prompted growth arrest and cell death of HCC cells. MS2 was also conjugated with IRGD peptide for targeted delivery of apoptosis-inducing Tl+ ions (Thallium), which caused apoptosis in human breast cancer cells MCF-7 and MDA-MB-231 as well as effected necrosis of these tumor xenografts in mice [103].

Bacteriophage Qβ is highly stable and has been used for ligands and proteins displaying on the exterior surface. Rhee et al. utilized Qβ VLPs to encapsulate multiple copies of fluorescent proteins by specific engineered RNA-protein interactions [104]. The resulting VLPs were attached with 9-biphenylcarbonyl (BPC)-sialoside moieties via CuAAC reaction, which showed selective entry into Chinese hamster ovary cells through the recognition between BPC-sialoside and CD22 receptor. PEGylation was employed to modify the surface of Qβ VLPs by the attachment of PEG via thiol-dibromomaleimide chemistry [23]. PEGylated Qβ VLPs were further installed with approximately 530 photocleavable DOX complexes for photocaging therapy by CuAAC reaction. The dual-functionalized VLPs showed negligible cytotoxicity before photoactivation, whereas highly controllable photo-release and cell killing power were also confirmed.

3.4. Other protein cage nanoparticles

In nature, heat shock protein (Hsp), ferritin, apoferritin (Apf), and other small proteins can also self-assemble into protein cage architecture like viral capsids [105]. These
spherical NPs can serve as drug delivery vehicles just like virus and VNPs.

Hsp derived from Methanococcus jannaschii was modified with cell-specific targeting moieties by both genetic and chemical strategies [106]. A tumor vasculature targeting peptide RGD-4C (CDCRGDCFC) and anti-CD4 antibodies were both incorporated onto the exterior of Hsp cage, which exhibited specific binding to CD4+ cells. Genetically modified Hsp cage was also loaded with DOX, showing selective release in a pH dependent manner [107].

Ferritin is an iron storage protein presented in all living systems, which self-assembles into a 24-mer capsid with a diameter of 12 nm [108]. Kanekiyo et al. exploited the ferritin-NP system to display Epstein-Barr virus (EBV) major envelope glycoprotein 350/220 (gp350), resulting in 10- to 100-fold increased neutralization compared with soluble gp350 in a mouse model [109]. As the iron-free form of ferritin, Afp can load molecular cargoes such as high dose of DOX and docetaxel (DOC), specifically bound and deliver them into MCF7 and HeLa cell lines, and enhance G2/M phase arrest and apoptosis compared to free drugs [110].

Bovine serum albumin (BSA) can comprise biocompatible nanoparticles and be constructed as nanocarriers for organic selenocompound (PSeD) decorated with FA as the targeting ligand [111]. The cancer-targeted nanosystem drastically increased reactive oxygen species overproduction, VEGF/VEGFR2 inactivation, and inhibition of XRCC-1-mediated repair of DNA damage in Hela cells. Encapsulin, isolated from thermophilic bacteria Thermotoga maritima, was genetically engineered with unusual heat stability and SP94 peptide [112]. Aldoxorubicin (AlDox) was chemically packaged inside SP94-Encapsulin via thiol-maleimide Michael-type addition for targeted delivery to HepG2 cells, displaying comparable killing efficacy with free AlDox. The thermostable E2 subunit of pyruvate dehydrogenase was utilized to construct approximately 27-nm dodecahedral protein NPs [113]. The internal cavity was loaded with Alexa Fluor 532 (AF532) and DOX, the external surface was shielded with PEG or FA, and the bi-functional NPs showed cell uptake six times greater than non-targeting NPs.

4. Applications of Viral Nanoparticles

4.1. Contrast agents for biological imaging

A contrast agent is a substance which can increase the imaging intensity of cellular components. A variety of contrast agents have been developed for optical imaging, ultrasound imaging, computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) [114,115]. In order to reveal biological processes with disease present in the living subjects, an ideal imaging contrast agent should have high intensity at the desired site with minimal toxicity [116]. Intensive research efforts have been made aiming to achieve a satisfactory signal-to-noise ratio. In this regard, VNPs provides more advantages over synthetic nanoparticles such as short circulation and retention time, possible evasion by the immune system, and negligible side effects [117]. Furthermore, VNPs can be engineered with diverse fluorescent labels and/or contrast agents along with targeting aptamers, peptides, or antibodies for specific binding with targeted cells and tissues [12].

The interior and exterior surfaces of MS2 were conjugated with gadolinium chelates (Gd3+) to fabricate MRI contrast agents [118]. The proton nuclear magnetic relaxation dispersion profiles of the conjugates showed up to a 5-fold increase in relaxation, with reduced systemic toxicity compared with free Gd3+ ion. Multifunctional PVX was constructed by loading Gd-dodecane tetraacetic acid to lyses for MRI and installing fluorescent dye O488 to cysteines for optical imaging [119]. The resulting nano-filament displayed significantly enhanced relaxivities as well as good fluorescent properties by showing no quenching, which could be used in dual-modal optical-MRI imaging applications. The internal cavity of TMV was loaded with a dysprosium (Dy3+) complex and NIR fluorescent dye Cy7.5, and the external surface was conjugated with an Asp-Gly-Glu-Ala (DGEA) peptide to target integrin αβ1 [120]. The resulting Dy-Cy7.5-TMV-DGEA NPs demonstrated a high transverse relaxivity for MRI and target specificity with PC-3 prostate cancer cells and tumors in vitro and in vivo.

The inside surface of MS2 have also been addressed with [18F]-fluorobenzaldehyde and evaluated in rats for PET imaging, which showed a much longer circulation time of over 3 h for [18F]-MS2 NPs compared with 0.5 h of free [18F]-fluorobenzaldehyde [121]. CPMV was fluorescently labeled with high densities of Alexa Fluor 555 (A555) without any measurable quenching, and the resulting exceptionally bright VNPs were assessed for intravitral vascular imaging [122]. CPMV-A555 exhibited high-resolution intravitral imaging of vascular endothelium for periods of at least 72 h and a depth of up to 500 μm in the vasculature and blood flow of living mouse and chick embryos. Moreover, the intravitral visualization of human fibrosarcoma-mediated tumor angiogenesis using CPMV-A555 offers a measure to evaluate arterial and venous vessels and to examine the neovascularization of the tumor microenvironment.
4.2. Gene therapy
Gene therapy is a prospective approach to treat myriad genetic and acquired diseases [123]. As the natural vehicles for delivery of genes, virus has the most advantage for therapeutic nucleic acids delivery. Currently about two-thirds of gene therapy in clinical trials are using viruses as transportation vectors, among which mammalian viruses are majority [124].

Ad and AAV vectors have been developed as delivery vehicles for CRISPR components by many research groups. Maggio and co-workers used early region 1 (E1) and early region 2A (E2A)-deleted Ad vector for the delivery of Cas9-protein/gRNA complexes into transformed and non-transformed cells, resulting targeted mutagenesis ranging from 18% to 65% across different human cell types [125]. Li et al. constructed a AAV8 mediated delivery of CRISPR-SaCas9 system compatible with hepatitis B virus (HBV) core region derived guide-RNA for the inhibition of chronic HBV infection, which showed declined levels of serum HBsAg, HBeAg, and HBV DNA in HBV transgenic mice during 58 days observation [126]. Yin et al. utilized lipid NPs for the delivery of Cas9 mRNA with AAV encoding a sgRNA and a repair template to repair human hereditary tyrosinemia in mouse model, showing fumarylacetoacetate hydrolase (Fah)-positive hepatocytes generated with the efficiency of > 6% of hepatocytes after a single Fah-splicing mutation [127].

Due to their capability to persist in humans after primary infection, HSV has been used as vehicles to delivery genes to cells in vivo [128]. HSV vectors have been successfully applied in animal models for the treatment against cancer, among which HSV-1 is the most extensively studied [129]. Lin et al. engineered CRISPR/Cas9-mediated editing of the HSV-1 genome, which demonstrated that two copies of ICP0 in different locations could be simultaneously edited with high efficiency and without off-target editions [130]. Roehm et al. utilized lentiviral-mediated transfection of CRISPR/Cas9 to target ICP0 combined with ICP4 or ICP27 in HSV-1 genome, causing complete inhibition of HSV-1 infection [131].

4.3. Drug delivery
Nanoparticles-based drug delivery can provide targeted and intracellular delivery, promote enhanced drug-therapeutic efficiency, and minimize required doses by reducing immunogenicity and increasing the solubility and specificity [132,133]. As the aforementioned examples, VNPs can also improve the bioavailability, enable controlled release upon physiological conditions, and deliver two or more therapeutic compounds for combination therapy.

To enhance the delivery and targeting efficiency of VNPs, several approaches were employed such as PEGylation and coating. PEGylation is a common strategy to reduce or eliminate biospecific interactions by the attachment of PEG [134]. PEGylated versions of VNPs have been constructed with CPMV, PVX, TMV, and MS2, which shielded their interactions with cells and prevented VNPs from triggering a primary immune response [135-137]. Although shielding efficiency is dependent on the PEG chain length, only minimal surface coverage (< 1%) is needed to block CPMV-cell interactions [135]. Similar data were also reported for PEGylated PVX and TMV formulations [136,137]. The coating of DNA origami nanostructures with CCMV capsid proteins through electrostatic interactions augmented cellular delivery to human HEK293 cells, which proved 13-fold higher for cellular attachment and delivery of origamis compared to bare DNA origamis [138].

4.4. Subunit vaccines
As the world is currently going through an epidemic of a novel coronavirus (2019-nCoV), we should think prudently how to deal with viruses and use them as a potentially vast and beneficial resource [139]. A most recent example was the repurposing of nonstructural protein 10 (NSP10) from the replicase polyprotein 1a of human severe respiratory syndrome (SARS) coronavirus to exploit a self-assembling platform for antigen presentation and vaccine development, e.g. the truncated Herpesvirus saimiri gD protein fusion-expressed with NSP10 as a therapeutic vaccine for idiopathic pulmonary fibrosis [140]. Viruses have been proven as useful development platform for novel vaccines because of their particulate and repetitive nature.

There are several viral vaccines against cancer commercially available now, including HBV and human papillomavirus (HPV) vaccines [141]. Zhai et al. developed an HPV vaccine candidate composed of MS2 VLPs displaying the mixture of different peptides of HPV capsid protein L2 [142]. The mixed MS2-L2 VLPs protected mice against eleven oncogenic HPV types associated with approximately 95% of cervical cancer. Speiser et al. constructed a therapeutic human VLP-based vaccine Qβ(G10)-Melan-A through installing the melanoma differentiation specific antigen Melan-A/Mart1 to Qβ VLPs packaged with the targeting TLR-9 ligand G10 (a type-A CpG) for DCs and CD8+ T cells [143]. Phase I/II study of Qβ(G10)-Melan-A in Stage II/IV melanoma patients showed promising results with 63% of the patients generating specific T cell responses to produce high cytokine levels (IFN-γ, TNF-α, and IL-2). Shukla et al. utilized CPMV for HER2 epitope delivery, revealing efficient lymphatic drainage, high uptake and activation with antigen presenting cells, and enhanced anti-HER2 immunity [93]. The CPMV-HER2 vaccine candidate postponed the progression and metastasis of DDHER2 primary tumors in Balb/c mice. Cai et al. compared three
Icosahedral plant VNPs of CPMV, CCMV, and sesbania mosaic virus (SeMV) for their capability of displaying HER2 epitope [144]. Although these obtained VNPs showed similar shape and structure, the immune profiles elicited by them were significantly different, whereas a Th1 predominant immune response was observed for CPMV-HER2 while a Th2 type for CCMV- and SeMV-HER2. The results demonstrated that the VNP carrier itself can play a key role in the regulation of Th1/Th2 differentiation.

5. Summary and Future Outlook

The application of VNPs in drug delivery has taken tremendous steps forward in last 20 years. In the field of drug delivery, contrast agents, gene therapy, and viral vaccines, numerous VNPs-based platforms are now under various stages of preclinical and clinical development [145]. The currently approved VNP systems have highlighted the therapeutic potential of anticancer drugs by improving drug efficacy or reducing drug toxicity, even overcoming the disadvantages of immunogenicity or biocompatibility [146].

However, there are still some concerns for pushing viral drug delivery research forward. The first and biggest obstacle is whether pre-existing or elicited immunity would be initiated with the virus administration that generally lead to adverse effects or limit therapeutic effects [147]. The second bottleneck is the large-scale manufacturing process and techniques associated with formulation quality control and assurance of viral products [148]. The last hurdle is the safety issue of VNPs should be thoroughly tested and examined for long period before clinical application [149]. With the introduction of safer nanomaterials and novel engineering approaches, the field of viral drug delivery will continue to grow and flourish, and more multifunctional nanoparticles may be expected to enter into clinical practice in the near future.

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Ethical Statements

The authors declare no conflict of interest.

Neither ethical approval nor informed consent was required for this study.

Nomenclature

| Abbreviation | Description |
|--------------|-------------|
| AAV          | adeno-associated virus |
| Ad           | adenovirus |
| AlDox        | aldoxorubicin |
| ASGPRs       | asialoglycoprotein receptors |
| Apf          | apoferritin |
| biRNA        | bifunctional linker RNA |
| βlac         | β-lactamase |
| BPC          | 9-biphenylcarbonyl |
| BSA          | bovine serum albumin |
| CCMV         | cowpea chlorotic mottle virus |
| CPMV         | cowpea mosaic virus |
| CT           | computed tomography |
| CuAAC        | copper(I)-catalyzed azide-alkyne cycloaddition |
| DOC          | docetaxel |
| DOX          | doxorubicin |
| E1A          | early 1 A adenoviral |
| EGFP         | enhanced green fluorescent protein |
| EGFR         | epidermal growth factor receptor |
| FA           | folic acid |
| Fah          | fumarylacetacetate hydrolase |
| GBM          | glioblastoma multiforme |
| GM-CSF       | granulocyte-macrophage colony-stimulating factor |
| HBV          | hepatitis B virus |
| HCC          | human hepatocellular carcinoma |
| HepG2        | human hepatocellular carcinomas |
| HER          | human epidermal receptor |
| HPV          | human papillomavirus |
| Hsps         | heat shock proteins |
| HSV          | herpes simplex virus |
| ICP6         | infected cell protein 6 |
| MCF7         | Michigan cancer foundation-7 |
| MHC          | major histocompatibility complex |
| Mo-MLV       | Moloney murine leukemia virus |
| MRI          | magnetic resonance imaging |
| MTO          | mitoxantrone |
| MV           | measles virus |
| NDV          | Newcastle disease virus |
| NHS          | N-hydroxysooceimide |
| NIR          | near-infrared |
| NIS          | sodium-iodide symporter |
| NPs          | nanoparticles |
| NSP10        | nonstructural protein 10 |
| OVs          | oncolytic viruses |
| PAA          | polyacrylamide |
| PEG          | polyethylene glycol |
PET : positron emission tomography
PhenPt : phenanthriplatin
PSeD : selenocompound
PVX : potato virus X
RCNMV : red clover necrotic mosaic virus
SARS : severe acute respiratory syndrome
scFv : single-chain antibody variable fragment
SeMV : sesbania mosaic virus
siRNA : small interfering RNA
tHBcAg : truncated hepatitis B virus core antigen
TMV : tobacco mosaic virus
VEGFR : vascular endothelial growth factor receptor
VLPs : virus-like particles
VNPs : viral nanoparticles

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