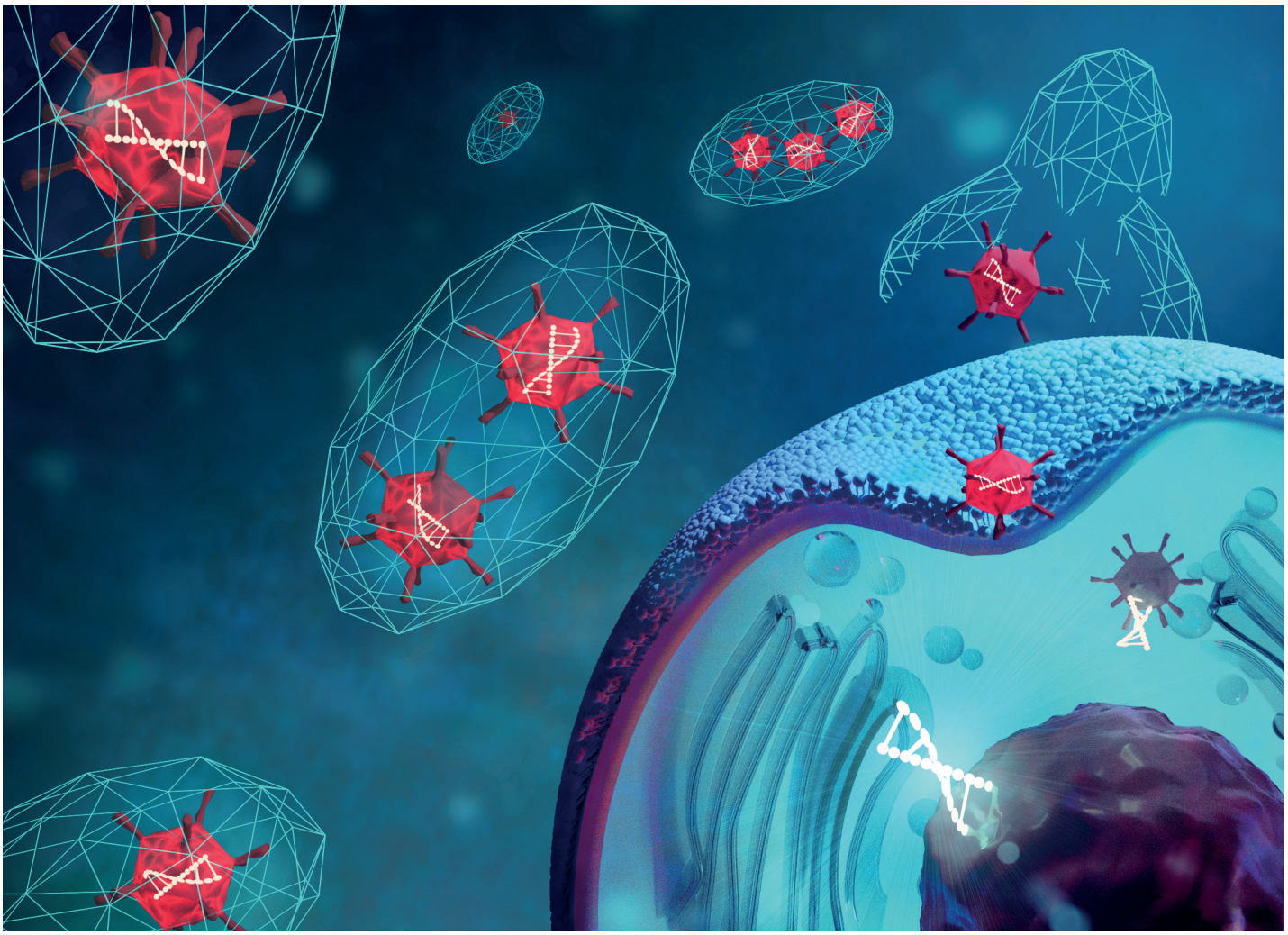

Showcasing a review article from researchers of the Max Planck Institute for Polymer Research, Mainz, Germany.

Materials promoting viral gene delivery

This review summarizes the progress in developing materials that enhance viral transduction, including polymers, peptides, lipids, nanoparticles, and small molecules, pointing out therapeutic applications in research and clinical context.

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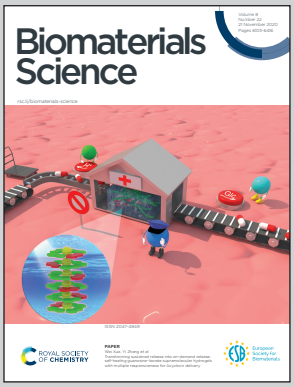

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Materials promoting viral gene delivery

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Therapeutic viral gene delivery is an emerging technology which aims to correct genetic mutations by introducing new genetic information to cells either to correct a faulty gene or to initiate cell death in oncolytic treatments. In recent years, significant scientific progress has led to several clinical trials resulting in the approval of gene therapies for human treatment. However, successful therapies remain limited due to a number of challenges such as inefficient cell uptake, low transduction efficiency (TE), limited tropism, liver toxicity and immune response. To adress these issues and increase the number of available therapies, additives from a broad range of materials like polymers, peptides, lipids, nanoparticles, and small molecules have been applied so far. The scope of this review is to highlight these selected delivery systems from a materials perspective.

The promise of viral gene delivery

Due to their inherent infectivity, non-pathogenic viral particles (VPs) are highly interesting vectors for gene delivery. In therapeutic applications they replace or deactivate disease-causing genes or introduce new genes to treat a disease.[1] Since the first commercial production in 2003[2] the amount of gene therapies entering clinical trials and eventually earning approval is growing each year[3,4] with promising future prospects.[5] Since 2017, the European Medicine Agency (EMA) and the U.S. Food and Drug Administration (FDA) approved eight new therapeutics,[6,7] amongst them for example Luxturna®, the first adeno-associated virus-based gene therapy[8] and recently Zolgensma® a one-time injection gene therapy to treat spinal muscular atrophy.[9]

Novel tailor-made gene therapeutic strategies pave the way towards personalized medicine, in which the patient's individual genetic circumstances are considered. Viral gene delivery promises to enable various new treatments and current research is directed at areas such as oncolytic virotherapy,[10] tissue regeneration and formation (bones,[11] spinal cord,[12]

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eye,[13] cardiac muscles[14]), treatment of hemophilia,[15] neurodegenerative diseases[16] and cystic fibrosis.[17]

Unfortunately, therapies in clinical studies have faced serious setbacks through side effects such as serious liver injuries.[9] A lethal case due to high dose adenovirus administration in 1999[18] raised attention to safety issues like immunogenicity, range of infectable host cells (tropism) as well to biological limitations, such as loading capacity of genetic information and batch-to-batch variation of viral vectors (VV).

Consequently, alternatives to VVs have become popular in the last 20 years. Non-viral gene delivery tries to mimic VVs by packaging genetic information in synthetic carriers like cationic polymers or lipids or by using physical methods for direct delivery to host cells as reviewed elsewhere.[19] The advantages of these approaches are a lower immunogenicity due to the lack of preformed antibodies and an easier targeted delivery owing to the modular design of synthetic carriers.[20] However, non-viral gene delivery methods often require more laborious preparation and suffer from low efficacy compared to viral methods.[21] Viruses, optimized through millions of years of evolution, still continue to be the most efficient gene carriers also for therapeutic approaches and are hence used in more than 70% of clinical trials[22] and in the majority of approved gene therapy drugs.[7]

Viral vectors

Viruses are highly ordered nanoassemblies of nucleic acids and proteins and can infect a broad range of organisms from bacteria to humans, both in pathogenic and non-pathogenic contexts.[23,24] Therapeutic VVs are obtained by replacing original gene sequences with beneficial ones.[25,26] These nucleic acids are packaged in a virus capsid, which is composed of proteins displaying functional groups like amines on the surface and can interact with specific receptors on cell membranes. Some virus capsids are additionally enveloped by a lipid bilayer.[27] The virus mediated delivery of nucleic acids (transduction) consists of several steps involving diffusion and translocation through the cell membrane, followed by disruption of the endosome (endosomal escape), release and integration of viral genomes to the nucleus.[26,28]

In clinical trials, adenoviruses (Ad) are the most frequently used VVs for gene transfer. Ad provides high transduction efficiency (TE) to a broad range of dividing and non-dividing cells.[29] Ad tropism is mainly determined by capsid proteins, hexones and fibers,[30,31] which contain fiber knob domains for coxsackievirus and adenovirus receptor (CAR) interaction.[32] The Ad serotype 5 (Ad5) is one of the most common vectors in clinical trials, however, they accumulate in the liver and show strong immunogenicity due to high prevalence of pre-existing immunity to Ad.[33]

Adeno-associated viruses (AAV) have emerged as promising vectors with low immunogenicity and long-term stability, resulting in the recently approved therapies, marketed as Luxturna® and Zolgensma®.[34,35]

As part of the retrovirus (RV) family, lentiviruses (LV) have been increasingly applied in recent years due to their inherent ability to infect both dividing and non-dividing cells, efficient integration into host genetic information and possibility for large scale production.[36–39]

Finally, oncolytic viruses are attracting growing interest as versatile cancer therapeutics.[40–45] They can selectively replicate in cancer cells, eventually inducing cell-lysis and further activating antitumor immune response.[46,47] Promising oncolytic vectors are genetically modified from adenovirus, herpes virus, reovirus and measles virus.[48–51]

The properties of the most common VVs discussed in this review are summarized in Table 1.

Limitations

Despite intense research efforts, VVs still face several limitations. An immune response by the host can lead to rapid inactivation of viral particles and clearance from the blood stream. Subsequent inflammatory processes can lead to liver injury or even multiorgan failure, in severe cases.[60,61] The production of neutralizing antibodies is a lasting humoral immune response, which hinders any further viral gene delivery.[62] To minimize an immune response, low vector doses are used which in turn results in reduced efficiency.

Moreover, the entry to cells can be limited by the natural tropism of VVs. For example, Ad cannot transduce to CAR-negative $CD34^+$ stem cells.[63] Further, transduction can also be hindered by low passive diffusion rates to cell membrane,[64] poor translocation to the cellular endosome, slow endosomal escape and inefficient nuclear genome integration.[65,66]

Strategies to promote viral gene delivery

Over several decades various techniques to promote the delivery of VVs have been developed. These can be categorized into (i) physical methods, (ii) genetic bioengineering of viruses, and (iii) chemical methods, namely material additives. The main aims of these delivery strategies include enhancing transduction efficiency (TE), long-term release of VPs, reduction of

Table 1 Overview of most common VVs for gene delivery studies

Name	Avg. R_h	Zeta potential	Enveloped	Genetic payload	Ref.
Ad	100 nm	−30 mV	No	37 kb	47, 52 and 53
AAV	29 nm	−9 mV	No	4.7 kb	54–56
LV	166 nm	−18 mV	Yes	8 kb	57,59

immune response, broadening of tropism and targeted delivery.[6,67]

Physical methods

Physical methods such as microinjection,[68,69] microfluidic,[70,71] sonication,[72,73] centrifugation,[74] cellular deformation,[75] laser irradiation[76,77] or electroporation[78,79] induce or facilitate cell entry of vectors by mechanical force, mainly through disrupting the plasma membrane. They are commonly applied in non-viral gene delivery because they transport the genetic information easily without the need for carriers and the otherwise low infectivity. These methods are also viable for viral gene delivery with electroporation being a noteworthy exception.[80] A downside of using VVs with physical methods is the lack of protection from immune response as well as the invasive and cell-damaging procedure. Very comprehensive overviews of membrane disruptive methods for cargo delivery have recently been published elsewhere.[81,82]

Genetic engineering

Genetic engineering of virus capsids is laborious but gives access to vectors with higher safety profile, modified tropism, and no batch-to-batch variability. Especially in recent years, highly efficient vectors could be obtained using genetic manipulation.

Strategies include amino acids mutations, peptide domain insertions and incorporation of chemical functional groups[83–85] as recently reviewed for AAV.[86–88] For example, introduction of unnatural amino acid residues such as azides on the capsid surface can give access to selective click chemistry.[89,90] Further, genetically modified viruses can enhance TE,[91,92] broaden the tropism,[63,93] enable fluorescent imaging,[85] escape neutralizing antibodies,[94,95] generate stimuli responsive vectors,[96] *e.g.* by light[97,98] or enzymes,[99] and target cells.[100,101] Comprehensive recent reviews on bioengineering AAV can be found elsewhere.[102,103]

Chemical methods

Viral gene delivery can be enhanced by synthetic additives mostly by promoting attractive virus–cell interactions. To this end, VPs are either chemically modified on the outer sphere or non-covalently bound to additives.

Covalent attachment of additives to virus capsid yields stable and irreversible modifications.[104] However, the covalent attachment might interfere with transduction and deactivate viruses by shielding epitopes on the capsid surface necessary for adhesion.[105]

Chemical modification of the virus surface requires mild conditions to maintain the integrity of VPs.[106] Due to the abundance of lysine in the capsid protein,[107] most bioconjugation methods are mild amine functionalizations at physiological conditions, *e.g.* using *N*-hydroxysuccinimide (NHS).[108] Other approaches apply azide–alkyne click chemistry[109,110] and utilize thiol-displaying bioengineered capsids for Michael addition.[111]

Non-covalent attachment is simpler and generally obtained *via* attractive electrostatic interactions or incorporation in various scaffolds. This can result in different architectures such as coatings,[112–114] complexes,[115–120] capsules,[121–125] and matrices[126–128] as illustrated in Fig. 1. Table 2 provides a summary of these architectures and highlights their respective benefits and drawbacks.

Enhanced interactions between cells, VPs and additives can be obtained *via* various pathways.

For cationic delivery agents, binding is mainly driven by attractive electrostatic interactions.[129] This results in directed diffusion and colocalization of viral particles and cell membranes, thereby facilitating cell entry. Some materials can further enhance the fusion of viral and cellular membranes.[130]

Cationic as well as anionic additives with high molecular weight can sediment with VPs onto cells in culture, which increases TE due to increased contact between VPs and cells.[131,132]

VVs can also be immobilized on surfaces prior to cellular adhesion.[133,134]

Chemically facilitated cell entry can also be achieved by manipulating the host cell itself. Especially small cytostatic molecules can affect viral infectivity and TE by intervening with cell cycle processes.[135]

Applying delivery systems able to bypass receptor mediated cell entry can broaden tropism. For example, CAR is downregulated in cancer cells, which makes oncolytic Ad delivery challenging.[136] Delivery systems can improve addressing VPs to CAR negative cells and circumvent CAR-mediated endocytosis.[116,137–140]

A drawback of chemical delivery systems are cytotoxic reactions due to high additive concentrations.[114,141] Furthermore, non-degradable additives, *e.g.* high molecular weight polymers can result in unwanted accumulation and health risks *in vivo*.[142]

Delivery systems

Viral gene delivery systems including polymers, peptides, lipids, nanoparticles, and small molecules display diverse modes of action on a wide range of size scales (Fig. 2). The main focus of this review is to highlight materials for promoting viral gene delivery.

Polymers

Polymers are the most extensively studied delivery systems for VVs. They typically consist of covalently connected repeating monomeric units and reach a molecular mass of several kDa. This section provides an overview on common and emerging polymeric materials of natural and synthetic origin.

Research on polymeric delivery agents began with DEAE-D as one of the first reagents used for VV delivery to mammalian cells in 1965.[143] Due to its advantageous ease of use and high transduction efficiency, it has long been a popular delivery agent for various virus types.[144,145] Nevertheless, one of

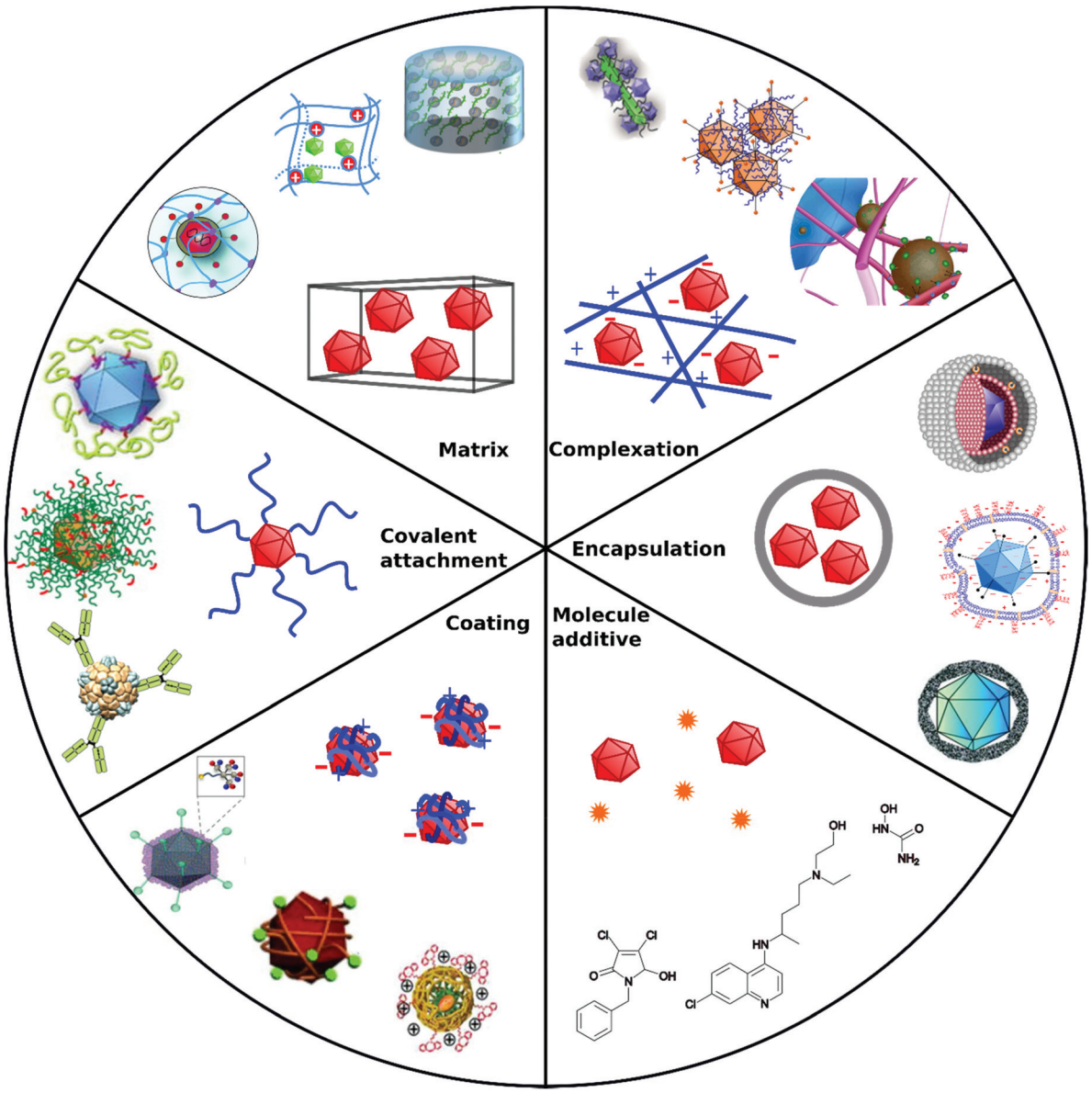

Fig. 1 Overview of different architectural formations for chemical delivery methods of VVs. Figure insets in clockwise order starting from top adapted with permissions from: ref. 274. Copyright 2014 The Royal Society of Chemistry, ref. 307. Copyright 2013 Elsevier, ref. 348. Copyright 2020 Springer Nature, ref. 421. Copyright 2019 American Chemical Society, ref. 422. Copyright 2014 Elsevier, ref. 112. Copyright 2012 John Wiley & Sons, ref. 208. Copyright 2019 John Wiley & Sons, ref. 209. Copyright 2012 John Wiley & Sons, ref. 254. Creative Commons BY 4.0. 2020 John Wiley & Sons, ref. 378. Copyright 2020 American Chemical Society, ref. 311. Copyright 2015 Elsevier, ref. 138. Copyright 2016 Elsevier, ref. 263. Copyright 2019 The Royal Society of Chemistry, ref. 291. Creative Commons BY 4.0. 2020 MDPI, ref. 282. Copyright 2012 Elsevier.

the major issues with DEAE-D is cellular toxicity at high concentrations needed for efficient transduction,[143] which resulted in a declining interest for its use as a delivery agent for VVs.

Beside DEAE-D, the cationic polymer polybrene was heavily used as an enhancer of viral delivery *in vitro* in early days.[146] Due to its cost efficiency as well as its simple and safe handling it remains a popular additive,[147] *e.g.* for enhancing delivery of Ad to human mesenchymal stem cells (hMSCs)[148] and as an additive in ultrasound-enhanced RV delivery to the retina.[72]

In general, it can be summarized that polycationic compounds like DEAE-D, polybrene, poly-L-lysine and protamine sulfate enhance TE, whereas polyanionic compounds like heparin, pyran or cyclodextrin modified with carboxylic groups inhibit TE and reverse the effect of polycationic additives, when combined.[146,149–151] However, anionic dextran sulfate in certain concentrations could promote focus formation of Rous sarcoma virus and thereby slightly enhance TE.[149] In contrast to these findings, dextran sulfate inhibited TE of HIV-1[152] and neutralized enhancing effects of cationic additives.[149] The anionic polysaccharide chondroitin sulfate C also showed

Table 2 Overview of chemical delivery approaches for VVs

Approach	Main formation pathway	Main function	Benefits	Limitations	Examples	Ref.
Covalent attachment	Bioconjugation chemistry on capsid proteins	Target cell receptors by specific capsid modifications, shield from immune response	Permanent, stable	Interference with capsid functions	Polymers, peptides,	104–106 and 108–110
Coating	Electrostatic interactions, precipitation	Overcome charge repulsion	Maintain virus capsid functions	Non-permanent, elimination due to cationic charge *in vivo*	Calcium phosphate, silica, flexible polymers	112–114
Complexation	Electrostatic interactions	Overcome charge repulsion	Maintain virus capsid functions	Non-permanent, elimination due to cationic charge *in vivo*	Peptide fibrils, iron nanoparticles	115–120
Encapsulation	Incorporation in capsules	Shield from immune response, overcome charge repulsion	Stealth VPs	Insulation of capsid functional domains	Liposomes, polymers	121–125
Matrix	Coincubation/ coincorporation/ cogelation	Local administration Implantable or injectable matrix	Spatiotemporal release	Degradability, unwanted accumulation	Hydrogels	126–128
Molecule additive	No formation with VPs	Manipulate cellular processes	Synergistic mode of action, easy to apply	Cytotoxicity, no directed VP diffusion to cell	Cytostatic drugs	135

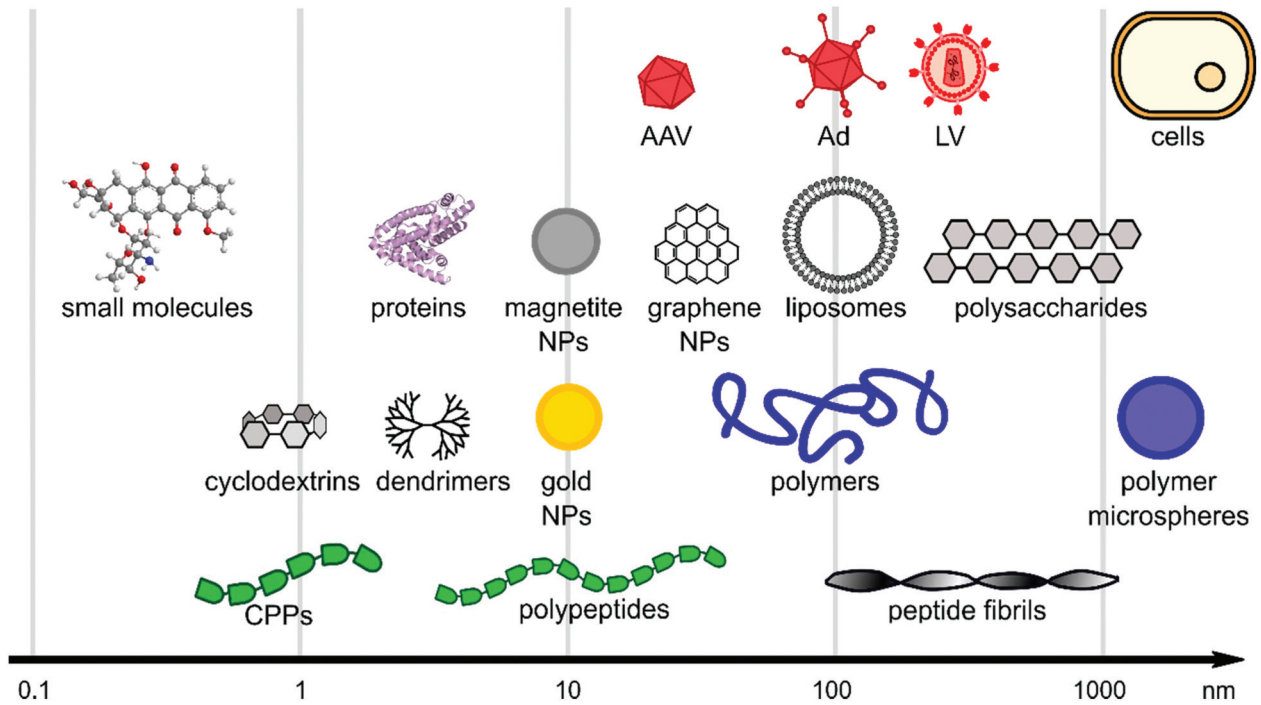

Fig. 2 Size scales of selected materials of interest for viral delivery. The *x*-axis is in logarithmic scale and schematic figures point out size range of material classes.

unexpected increase of TE in combination with polybrene *in vitro*. This observation was explained by sedimentation of larger polymer–virus complexes on cells in contrast to free diffusion of unmodified viruses in the supernatant solution.[131,153]

In order to yield VVs with improved safety profile, *e.g.* protect them from neutralizing antibodies and evade immune response, polymers have been coated[154,155] or covalently bound[156–158] to viruses. The safety of polymer-coated VVs has been increased by changing tropism and retargeting cells.[156] Further, natural and synthetic hydrogels from polymers have been investigated for spatially controlled VV delivery.[159,160] Amongst them macroporous hydrogels were utilized for long-term release of VVs,[161–164] and thermoresponsive polymer gels were applied as injectable systems.[127,163] A selected overview of general structural motifs of the polymers and a summary of all

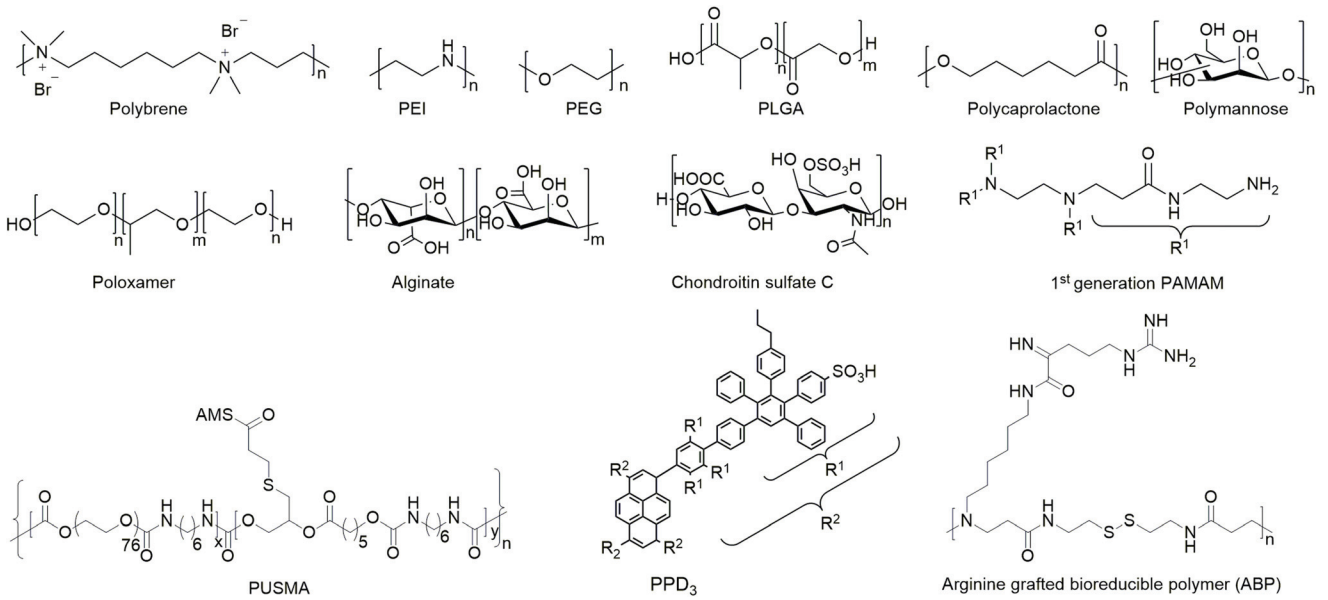

Chart 1 Overview of general structural motifs of selected polymers.

polymers discussed in the following subsections are provided in Chart 1 and Table 3, respectively.

PEG. Polyethylene glycol (PEG) is one of the most versatile polymers used in vast technical and consumer related areas due to its stability and biocompatibility.[165] For more than half a century, the pharmaceutic industry has employed PEG for shielding proteins from humoral immune response to prolong circulation time.[166–169] Consequently, viruses coated with PEG (PEGylated) were first developed to circumvent the immune response *in vivo*. An extensive review of PEG utilized for viral delivery was published in 2010.[170]

Covalent attachment of functional PEG derivatives to Ad capsid proteins, first introduced by O'Riordan in 1999,[106] protected the virus from neutralizing antibodies[106,157] and displayed reduced clearance rates by Kupffer cells.[171] However, PEG-coated stealthy VPs are hindered in cell membrane interaction, which can be tackled by bifunctional multipurpose PEG linkers. Cell specific bioactive peptides attached to these PEG linkers introduced targeted high affinity interaction while showing low immunogenicity even in systemic administration.[172,173] Protection from neutralizing antibodies has been shown for PEGylated AAV at certain cross-linking ratios.[174] Interestingly, TE of VPs varied depending on the grafting efficiency of the activation agents for covalent attachment of PEG and their susceptibility to induce aggregation of VPs.[175] Genetic modifications of AAV resulting in expression of thiol groups on the capsid surface extended bioconjugation methods with thioether or disulfide bonds, *e.g.* for selective conjugation of NHS-functionalized PEG.[83] Moreover orthogonal conjugation of PEG derivatives can also be achieved by click chemistry, when genetically modified AAV capsids presenting azide moieties are used.[176]

PEGylated Ad with low molecular weight of 5 kDa were successfully applied for accumulation in tumor tissue and showed enhanced permeability and retention (EPR) effect when injected intraveneously.[177] Noteworthy, in other studies, PEGylation of Ad with 20 kDa PEG instead of 5 kDa resulted in reduced liver transduction and hepatotoxicity.[178,179] PEGylation also shields Ad from pre-existing Ad antibodies. This is relevant for applications in vaccines or gene therapy due to possible pre-existing immunity against Ad in humans.[180] *In vitro* TE of PEGylated Ad was reduced compared to *in vivo*. This observations was traced back to higher cellular uptake with integrin and proteoglycan interaction, which is more strongly induced by pharmacodynamic force *in vivo*.[181] Beside neutralization of Ad by the serum, blood cells can also inhibit Ad activity after systemic administration.[182] Addressing this need, PEGylated Ad has been used to circumvent endothelial cell activation from blood cells and thereby improve TE and safety.[183]

However, a crucial issue is the so-called PEG-dilemma. Concurrent to EPR effect, PEGylated viruses show lower cellular uptake and lower endosomal escape eventually leading to reduced TE. In order to overcome this problem, PEG was combined with other polymers as reviewed elsewhere for non-viral gene delivery.[184] Copolymers from PEG include spherical poly-DL-lactide particles,[121] pH-sensitive poly(L-histidine-*co*-L-phenylalanine),[185] micellar poly(disulfide amine),[186,187] degradable gelatin[161] to name just a few examples. Fan *et al.* reported an elaborate design of a β-cyclodextrin–PEI-MMP-cleavable-peptide–PEG polymer (CDPCP, Fig. 3). This polymer design aimed to overcome liver accumulation by using PEG 5 kDa, and further to electrostatically coat Ad by grafting PEG onto a high-molecular weight, branched PEI-cyclodextrin copolymer *via* matrix metalloproteinase (MMP) sensitive peptide linkers

Table 3 Overview of polymer sequences, properties, and VP-polymer fabrication methods for the presented polymeric delivery systems

Abbreviation	Compound	Properties	Fabrication of VP-peptide	Ref.
DEAE-D	Diethylaminoethyl-dextran	c., h.	Inc. (poliovirus/avian sarcoma virus/simian virus 40)	143, 149, 144, 149 and 145
Polybrene	1,5-Dimethyl-1,5-diazaundecamethylene polymethobromide	c.	Inc. (sarcoma virus/RV/Ad)	146, 147 and 148
PEG	Polyethylene glycol	h.	Cov. conj. (Ad/AAV)	157, 173, 177, 178, 83, 174 and 176
PELA	Poly-DL-lactide-poly(ethylene glycol)	Amph.	Inc. in microspheres (Ad)	121
PEG*b*PHF	Poly(ethylene glycol)-*b*-poly(L-histidine-*co*-L-phenylalanine)	pH sensitive, h.	Inc., nanoplex formation (Ad)	185
Poly-histidine/PEG		h., pH sensitive	Inc. (AAV)	282
HA–PEG	Hyaluronic acid cross-linked with PEG	Porous scaffold	Inc. (LV)	162
CDPCP	β-Cyclodextrin–PEI-MMP-cleavable-PEG (MMP-cleavable = GPLGIAGQC)	c.	Inc. (Ad)	138
APP	PEGylated and taxol-conjugated polymeric arginine grafted poly(disulfide amine)	c., h.	Inc. (Ad)	187
PEGDA/PLL	Poly(ethylene glycol) diacrylate blended with PLL	h. matrix	Inc. (LV)	283
PEI	25 kDa poly(ethylene imine)	c.	Inc. (Ad)	114
PEI–DEG-bis-NPC	2 kDa PEI cross-linked with diethylene glycol	h., c.	Inc. (Ad)	200
PEI–CyD–FA	600 Da PEI cross-linked with cyclodextrin and folic acid	c., h.	Inc. (Ad)	199
PEI–DA	PEI conjugated with deoxycholic acid	c., h.	Inc. (Ad)	207
rPEI	1.8 kDa PEI cross-linked with cystamine	Branched polymers	Inc. (Ad)	196
PCDP	PEI cross-linked with cystamine derivative	Bioreducible, c.	Inc. (Ad)	203
PEI–pGMA	PEI-*b*-poly(glycidyl methacrylate)	c.	Inc. (LV)	210
PEI–DPA	PEI functionalized with 3-(3,4-dihydroxy-phenyl) propionic acid (catechol groups)	c., h.	Inc. (AAV)	209
PEI–DBCO	1.8 kDa PEI–dibenzocyclooctyl	c., h.	Cov. conj. (LV)	208
PEI/hyaluronic acid		c., a.	Layer by layer deposition (Ad)	201
PEI/chondroitin sulfate		c., a.	Layer by layer deposition (measles virus)	202
PEG-*g*-PEI	2 kDa PEG grafted on 25 kDa PEI	c.	Inc. (Ad)	139
Poloxamer 407	PEO_{101}–PPO_{56}–PEO_{101}	Viscous oil/gel	Inc. (Ad/LV/AAV)/ coinjection (Ad/LV)	230, 232, 233, 238, 229, 231 and 160
Poloxamer 407/ polycarbophil		Viscous oil/gel	Inc. (Ad)	239
Poloxamer PF68 and T908		Viscous oil/gel	Inc. (AAV)	236 and 237
LentiBOOST	Poloxamer 338 (PEO_{141}–PPO_{44}–PEO_{141})	Viscous oil/gel	Inc. (LV)	240–245
PAMAM, EGFR targeting peptide, PEG	Polyamidoamine G3, G4, G5, peptide: CYHWYGYTPQNVI, 2 kDa PEG	c., dendrimer	Inc. (Ad)	140
PAMAM, antibody, PEG	PEGylated polyamidoamine G4, 63 kDa, Erbitux®	c., dendrimer	Inc. (Ad)	251
PPD3	Polyphenylene 3	Amph., dendrimer	Inc. (Ad)	116
PPD3-dendron	One quarter of amphiphilic polyphenylene 3	Amph. dendron	Inc. (Ad)	254
PCL	Poly(ε-caprolactone)	Nanofibers	Electrospinning (Ad)	164
PCL/ELP	PCL blended with elastin like pentapeptide $(VPGVG)_{128}$	Nanofibers	Electrospinning (AAV)	223
PCL–AAV protein tagged	80 kDa PCL tagged with AAV protein binding to AAV	Microspheres or electrospun fibers	PCL–AAV protein binding	224
PLGA	Poly(lactide-*co*-glycolic acid)	p. (LA)/h. (GA)	Enc. (Ad)/lyophilization (LV, Ad)	155, 133 and 215
PLL/PLGA	Poly (lactic-*co*-glycolic) acid and poly-L-lysine		Inc. in PLL and enc. with PLGA (Ad)	213
PLGA/PLL	75/25 DL-PLGA 9.4 kDa/PLL 56 kDa	p. (LA)/h. (GA), cat., h. (PLL)	Inc. (Ad)	213
PLGA/PEG	50/50	p. (LA)/h. (GA), h. (PEG)	Cov. conj. of PEG–SPA and enc. in PLGA (Ad)	214
Alginate	Linear copolymer of $[$D-mannuronate (β1→4) L-guluronate (α1→4)$]_n$	h. gel	Emulsion (Ad)/cogelation (Ad)/cogelation (AAV, LV)/ cogelation (LV)	258, 259, 294 and 260–262
Alginate/poloxamer 407			Enc. (rAAV)	122
Chitosan	Poly β-(1→4)-linked D-glucosamine	c., linear polymer	Inc. (MLV)	269
Chitosan/β-glycerol phosphate	Crosslinked hydrogel	p., c.	Cogelation (LV)	127
Polymannose	Poly (1→6)-linked α-D-mannose	Linear and branched polymer network	Cov. conj. oxidative *via* $NaIO_4$ (Ad)/cov. conj. reductive amination (Ad)	266 and 265

Table 3 *(Contd.)*

Abbreviation	Compound	Properties	Fabrication of VP-peptide	Ref.
Cellulose-*g*-P (QDMAEMA)	Cellulose-grafted poly(*N*,*N*-dimethylaminoethyl methacrylate)	c., brush polymer	Inc. (cowpea chlorotic mottle virus and norovirus-like particles)	274
Polyaminoglycoside	Poly hydroxyethyl disulfide diglycidyl ether and tobramycin	Branched c. polymer	Inc. (LV)	276
β-Cyclodextrin		h. and p.	Cov. conj. (TMV)	272
α-Cyclodextrin	α-Cyclodextrin with pluronic PF68 and chondroitin sulfate or hyaluric acid	c., gel mixtures	Inc. (rAAV)	271
EGDE 3,3′	Ethylene glycol diglycidyl ether (EGDE) and 3,3′-diamino-*N*-methyl dipropylamine (3,3′)	Branched c. copolymer	Inc. (Ad)	295
Polydopamine			Inc. (AAV) on upside down polydopamine coated surface	290
Catecholamines	Polynorepinephrine or polydopamine		Seeding onto surface (AAV)	289
pHPMA	Poly(*N*-(2-hydroxypropyl)methacrylamide)	Polyvalent, h.	Inc. (Ad)/cov. conj. (Ad)	280, 156, 158 and 279
PUSMA	PEG cross-linked with 1,6-hexamethylene diisocyanate and epsilon caprolactone sulfamethazine	Biodegradable, thermoresponsive sol–gel polymer	Inc. (Ad)	163
Poly-arginine-*g*-polydisulfide amine		c.	Inc. (Ad)	114
Polyester urethane urea	Copolymer of polycaprolactone diol, butyl diisocyanate, and putrescine blended PEG	Nanofibers	Electrospinning (AAV)	222
Polystyrene	Polystyrene coated with methyl methacrylate and divinylbenzene	Nanocups	Inc. (VV)	293
pNaSS	Poly(ε-caprolactone) grafted poly(sodium styrene sulfonate)	c. film	Inc. and immobilization (Ad)	225
HEMA/APMA	Hydroxyethyl methacrylate (HEMA) with aminopropyl methacrylamide (APMA)	c. hydrogel	Inc. (rAAV)	291
PVA–VEA	Vinyl ether acrylate-functionalized poly(vinyl alcohol)	pH degradable hydrogel network	Inc. (Ad)	292
Poly(2-ethyl-2-oxazoline)		Thermoresponsive, h.	Cov. conj. *via* EDC/NHS (hepatitis B VLP)	278
Polyketal	Cross-linked amino ketal methacrylamide and ketal bis methacrylamide mixed with siRNA	Acid degradable	Polymerized on Eosin-5-isothiocynate conjugated AAV	284 and 285
DNA-aptamer	sgc8-aptamer	Polynucleotide	Cov. conj. (AAV)	288
	5′-ATCTAACTGCTGCGCCGCCGGG AAAATACTGTACGGTTAGA-3′	Polynucleotide	Cov. conj. (MS2 bacteriophage)	286
	Example: TGTGCCAAAGAGAGTGGTGGGGGGGTGG-GCGGAACTCGCG	Polynucleotide	Inc. (vesicular stomatitis virus)	287

Abbreviations: cationic (c.), hydrophilic (h.), hydrophobic (p.), amphiphilic (amph.), anionic (a.), incubation (inc.), encapsulation (enc.) covalent conjugation (cov. conj.), adenovirus (Ad), adeno-associated virus (AAV), lentivirus (LV), retrovirus (RV), tobacco mosaic virus (TMV), murine leukemia viruses (MLV).

(GPLGIAGQC). These peptides are cleaved in the environment of the tumor and uncover positive charge of PEI thus enhancing cellular uptake and Ad gene delivery. Thereby CDPCP avoids liver accumulation, inhibits Ad blood cell interaction and prolongs circulation time.[138]

These advances achieved in stimuli-responsive targeted delivery have the potential to be a valuable tool for systemic administration of viruses, *e.g.* of oncolytic viruses to tumor tissue, in future clinic applications. However, despite extensive use of PEG as a delivery system, in the 2010s, unexpected immune response and accelerated blood clearance of PEGylated therapeutics were reported.[188,189] Consequently there is a need for alternative polymeric delivery systems.[190]

PEI. Polyethylene imine (PEI), commercialized as jetPEI®, is a commonly used transfection agent in non-viral gene delivery and enhances TE by assisting cellular uptake and endosomal escape.[191,192] The secondary and tertiary amines in the backbone of the polymer yield positive charges at physiologic pH and promote attractive electrostatic interactions with anionic particles. After cell intake PEI can deliver its cargo directly into the cytoplasm by accelerated endosomal rupture without entering the degradative lysosomes due to its proton sponge effect induced buffering capacity and osmotic swelling at lower intracellular pH.[193–195] PEI can be applied in linear or branched forms for viral delivery.[196] One major drawback especially of high molecular weight PEI (25 kDa) is its cytotoxicity.[114,141] Attempts have been made to overcome this disadvantage by copolymerization. Grafting PEG onto PEI,[139,197] cross-linking with cyclodextrin,[198,199] introducing bioreducible disulfide groups[196] or diethylene glycol[200] improved toxicity, hemolytic activity, and enhanced transduction of Ad to CAR-negative cells. Lower immunogenicity was achieved through approaches such as layer-by-layer deposition of PEI with hyaluronic acid[201] or chondroitin sulfate[202] and by cross-linking PEI with cystamine.[203]

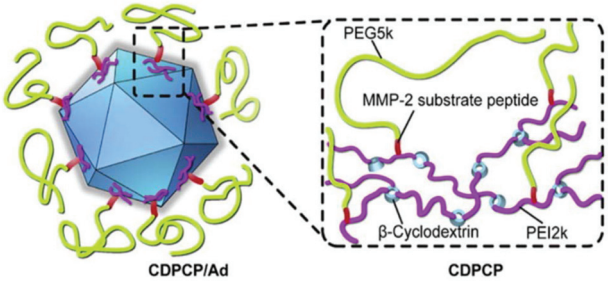

Fig. 3 Schematic diagram of Ad conjugated to enzymatically cleavable PEG–PEI–β-cyclodextrin (CDPCP). Adapted with permission from ref. 138. Copyright 2016 Elsevier.

Moreover cholesterol conjugated to PEI can facilitate cell entry *via* an energy- and endocytosis-independent membrane translocation pathway, originally known from cell penetrating peptides.[204,205] The amphiphilic combination of bile acids like deoxycholic acid and the cationic PEI efficiently transport cargo[206] and further enhance TE of Ad in CAR negative cells.[207]

An elaborate approach of PEI assisted gene delivery was applied by Pan *et al.* for genetic manipulation of primary T-cell to target malignant tissue as new potent immunotherapeutics. In a stepwise process, they first labelled T-cells with azide groups by metabolic incorporation of azide-glucose onto the membrane, then adding PEI-dibenzocyclooctyl-complexed LV. Conjugation of azide-dibenzocyclooctyl *via* click chemistry resulted in colocalization of complexed LV with T-cells, which facilitate interaction and robustly enhance TE in T-cells (Fig. 4A). *In vivo* experiments with tumor-bearing mice displayed significantly longer survival times than the control group or polybrene-assisted delivery.[208]

Delivery systems with spatially resolved gene expression have the potential to mimic the complexity of biological systems. An interesting example is branched PEI, which was functionalized with catechol groups and coated onto AAV. In this way, viral particles were obtained which could be placed and immobilized on tissue culture plates by a micropipette (Fig. 4B). Increased amounts of polymer coatings led to more stable immobilization resisting several washing steps and allowing to pattern viral particles. Thus, spatially resolved gene expression and cell manipulation could be achieved.[209] Recently, spatially defined transduction has been further simplified by coating poly(glycidyl methacrylate) copolymerized PEI as viral affine substrate. LVs were immobilized simply *via* electrostatic interactions and colocalized with HUVECs by incubation on this platform, resulting in enhanced TE and maintained cell functions (Fig. 4C). Further, patterned transduction and GFP expression could be generated by covering the substrate with a mask during fabrication.[210]

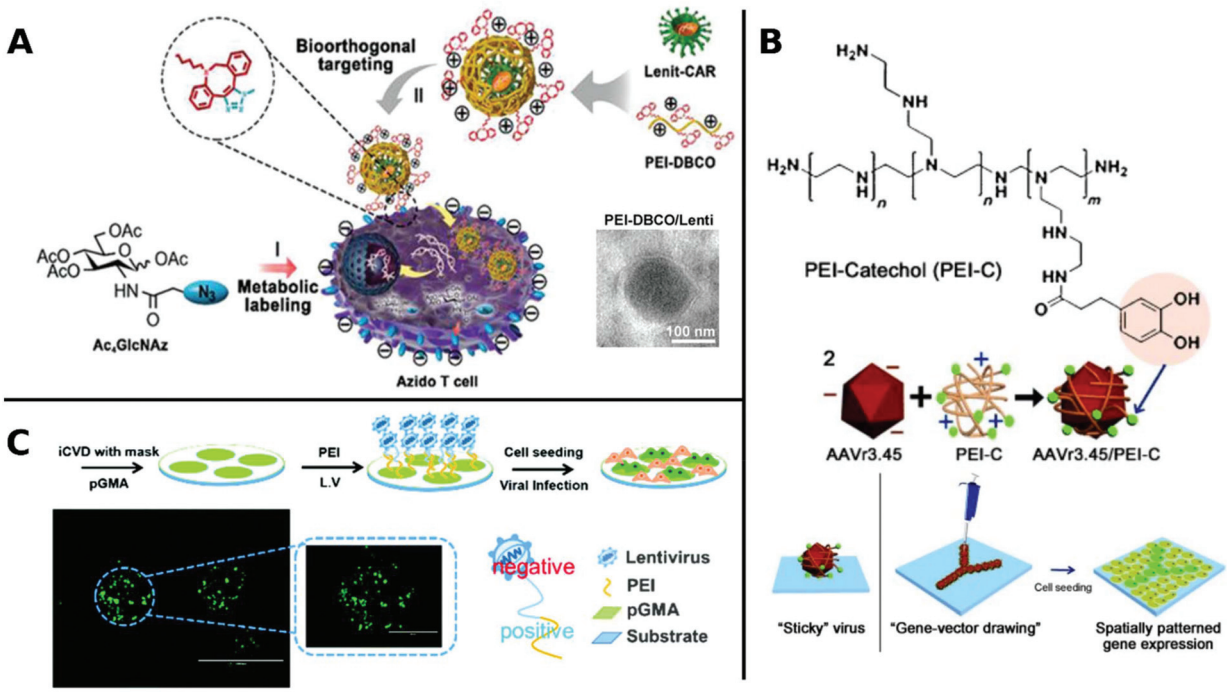

Fig. 4 A. Schematic illustration of PEI-DBCO/azide-glucose system in T-cells. LV are coated with PEI-DBCO, which is conjugated to azide-glucose pretreated T-cells *via* click-chemistry. Inlet TEM-image shows morphology of coated LV (scale bar: 100 nm). Adapted with permission from ref. 208. Copyright 2019 John Wiley & Sons. B. Formation of AAV/PEI-C hybrid particles and schematic illustration of immobilization and patterning of sticky vectors. Adapted with permission from ref. 209. Copyright 2012 John Wiley & Sons. C. Schematic fabrication process of viral delivery platform by subsequently coating of pGMA, PEI and LV attached by electrostatic interactions. Representative image of spatially patterned GFP-expression of transduced HUVECs (scale bar: 200 μm, inlet image: 400 μm). Adapted with permission from ref. 210. Copyright 2019 The Royal Society of Chemistry.

In recent years, PEI has evolved from a simple polymeric additive to a popular element in copolymeric formulations for enhancing gene delivery, as copolymers can mediate inherent drawbacks of PEI such as high toxicity.

Polyesters. Poly(lactic-*co*-glycolic acid) (PLGA) is an FDA approved copolymer of lactic acid and glycolic acid and commonly used as microspheres for controlled release of drugs and in tissue engineering.[211] PLGA encapsulation of cargo is typically achieved by an emulsion process without the need for charge induced adhesion.[211] However, cationic stabilizers like chitosan and PLL facilitate encapsulation by inducing attractive electrostatic interactions between negatively charged cargo, such as VPs, and PLGA.[212,213]

While Ad encapsulated in PLGA displayed greater cell viability and lower immunogenicity *in vivo*, it did not enhance TE.[155] The TE could be enhanced by PEGylation[214] before the encapsulation step and by surface modification of PLGA spheres with polysaccharides.[12]

In a tissue engineering approach, LV or Ad was immobilized in a PLGA scaffold and ensured long-term transgene expression over a period of four weeks *in vivo*. LV or Ad were incorporated in the PLGA scaffold by consecutive lyophilization of both components together with sucrose. Additional fibronectin and collagen could further enhance binding but not TE for LV delivery.[133] Zhu *et al.* incorporated Ad in PLGA scaffolds for bone regeneration *in vivo*. The electrospun PLGA yielded a nanofibrous scaffold, which enabled spatially defined, long-term gene expression of Ad encoding hBMP2 to promote osteogenic differentiation. This technique was able to cover more than 80% of the bone defects that could not be repaired in the control groups within eight weeks.[215] Another electrospinning approach coated oncolytic vaccinia virus onto PLGA nanofibers to maintain their antitumor activity at the tumor tissue site.[216] Furthermore, melt-processing was reported as a solvent- and additive-free approach for fabrication of PLGA–VP scaffolds. This homogenous dispersed composite materials for prolonged VP release are created by heating up PLGA and VP.[217]

Another example of polyesters is polycaprolactone (PCL) which is widely used as long-term implants in biomedical applications due to their good mechanical properties, slow degradability and biocompatibility.[218] In recent years, electrospinning techniques emerged as a promising process to create PCL fibrous structures for sustained and localized gene delivery.[219,220] In order to incorporate cargo to the fibers, coaxial spinning techniques can be utilized, in which an inner jet with cargo molecules and outer jet with polymer solution overcome the limitations of single stream electrospinning.[221]

In one such approach, Ad was encapsulated in PCL in a core–shell fashion for subsequent porogen-mediated release. Pores of approx. 3 μm size were created by leaching out incorporated low molecular weight PEG particles. The fibrous scaffolds enabled efficient local TE and reduced macrophage activation to attached cells.[164] Similar results were obtained with PCL containing copolymers of polyester urethane urea and polyester ether urethane urea electrospun together with low and high molecular weight PEG and AAV to yield various fibrous nanostructures for sustained delivery to cardiac tissue over a period of 2 months *in vitro* (Fig. 5A).[222]

Blending PCL with elastine-like pentapeptide (ELP) together with AAV resulted in nanofibrous scaffolds with adjustable degradability dependent on the polymer ratio for controlled AAV release.[223] Moreover, cysteine tagged proteins binding selectively to AAV, were attached to maleimide-displaying PCL microspheres or electrospun fibers and gave access to a therapeutic platform for intramuscular injection or subcutaneous implantation with reduced off-target (Fig. 5B).[224]

Recently, poly(ε-caprolactone)-grafted poly(sodium styrene sulfonate) films immobilized rAAV and enhanced gene delivery to hard-to-transduce hMSCs *in vivo*, thus enabling less invasive, effective treatment of focal cartilage lesions.[225]

PLGA and PCL were successfully applied as highly versatile platforms mainly as electrospun fibers and microspheres with controllable spatiotemporal gene delivery, especially considering applications in tissue engineering. Due to its biocompatibility and slow degradation rate both materials, PLGA and PCL has proven to be a reliable scaffold and a promising material especially for local, long-term gene delivery *in vivo.*

Poloxamers. Poloxamers, or Pluronics as a trade name, are commercially available, FDA-approved, thermoresponsive ABA-type triblock copolymers PEO–PPO–PEO consisting of two blocks of hydrophilic poly(ethylene oxide) (PEO) and one block of hydrophobic poly(propylene oxide) (PPO).[226]

Poloxamers are sold in various block lengths and commonly used in pharmaceutical and cosmetic applications as surfactants with no safety concerns.[227] More recently, poloxamers have also been applied as gels for gene delivery, as they exhibit thermal gelation behavior at concentrations higher than 20%.[228] For example, suspensions of VPs cooled at 4 °C can be gelated at physiological temperature around 37 °C, which gives access to homogeneous, injectable, spatially precise viral delivery *in vivo*.[229] However, a potential adverse effect of poloxamer injection concerning the temporal accumulation of microclots in vessels and organs has been reported.[142]

Several studies have shown enhanced TE of localized viral delivery by poloxamer 407 (PEO_{101}–PPO_{56}–PEO_{101}) amongst others to vascular smooth muscle cells *in vitro*,[230] to arteries *in vivo*,[231] to the central nervous system,[232] to solid tumors *in vivo*,[229] and for treatment of spinal cord injury.[160]

Rey-Rico *et al.* applied a series of poloxamers for enhanced AAV delivery to hMSCs even in the presence of host cell receptor binding inhibitory heparin or VP neutralizing antibodies. Prior to gelation, the VPs were encapsulated in poloxamer micelles to shield from adverse conditions, and cell viability was maintained to 100% over a period of 21 days during gene transfer in culture.[233] Long-term expression of AAVs to hMSCs have previously been reported for fibrin or RAD16-1 encapsulated viruses for shorter periods compared to poloxamer encapsulation.[234,235] Linear poloxamer PF68 and four branched poloxamer T908 encapsulated rAAV in a micellar architecture and enhanced spatiotemporal gene delivery efficiency to osteoarthritis chondrocytes *in vitro.*[236,237]

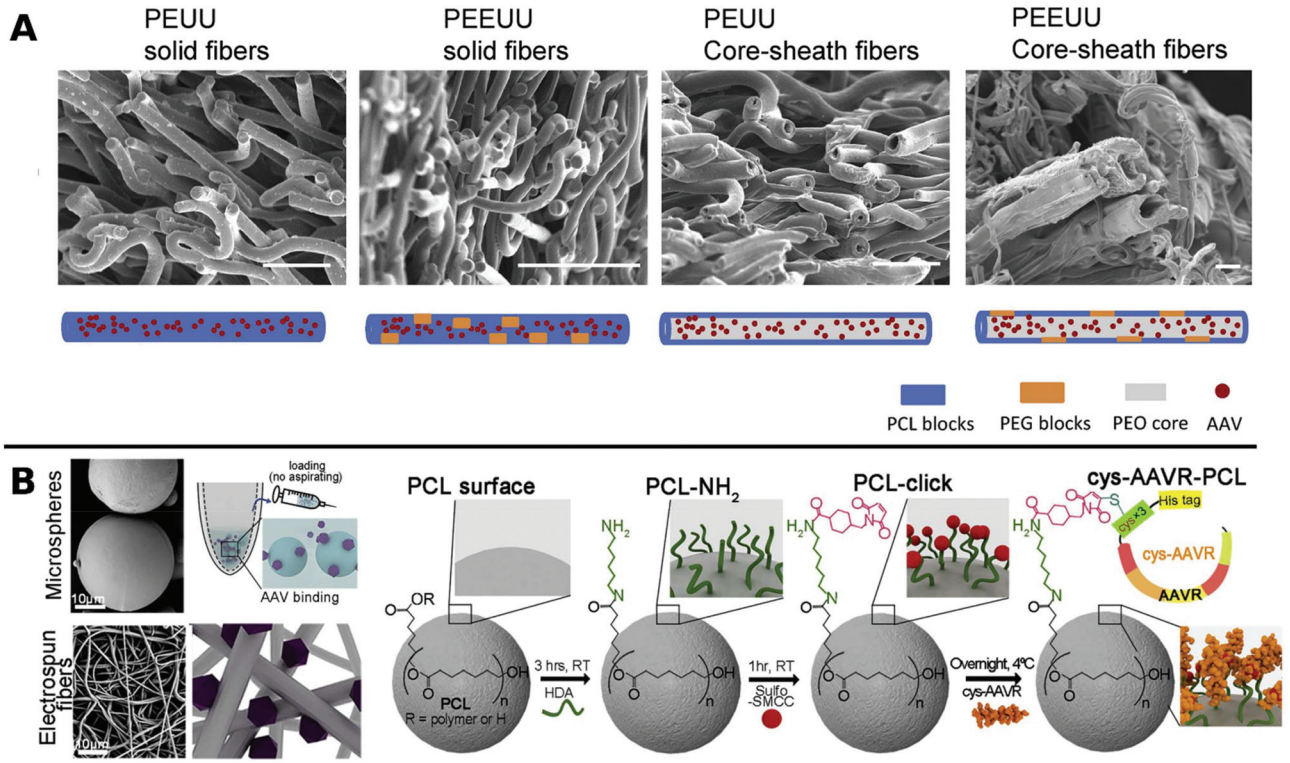

Fig. 5 A. SEM images at the cross sections of freeze dried polyester urethane urea (PEUU) and polyester ether urethane urea (PEEUU) scaffolds and schematic illustration of rAAV encorporation strategy (scale bar: 10 μm). Adapted with permission from ref. 222. Copyright 2017 Elsevier. B. SEM images representing PCL microspheres (top) and electrospun fibers (bottom) (scale-bar: 10 μm) with schematic diagram of AAV–PCL complexation. Reaction scheme shows steps to fabricate AAV tagged PCL. Adapted with permission from ref. 224. Copyright 2019 Elsevier.

Recently, treatment of cartilage damage was applied in a clinically relevant *in vivo* minipig model with rAAV delivered by poloxamer 407 (Fig. 6). Controlled release of the therapeutic SOX9 from the poloxamer gel improved repair of full-thickness chondral defects.[238]

To further enhance adhesiveness of poloxamer 407 for local viral delivery to organ surfaces, it was blended with >1% polycarbophil, a polyacrylic acid cross-linked with divinyl glycol. Addition of polycarbophil changed the sol–gel transition temperature and led to higher adhesiveness due to numerous carboxylic groups, which can easily form bonds with surrounding molecules. Spatially resolved and stable Ad delivery to heart tissue was shown *in vitro* and *in vivo* without having adverse impact to TE or cell viability.[239] Poloxamer 338 (PEO_{141}–PPO_{44}–PEO_{141}), also traded as synperonic F108, enhanced LV delivery in difficult-to-transduce T-cells more efficiently than polybrene. In combination with polybrene the TE was further elevated, which was explained by the distinct modes of each adjuvant, polybrene compensating electrostatic repulsion and poloxamer 338 fluidization the membrane.[240]

More recently, poloxamer 338 was commercialized as LentiBOOST™, and applied for LV delivery to CD34+ stem cells,[241–243] CD4+ and CD8+ stem cells,[244] and T-cells.[245] Enhanced TE, non-toxicity and clinical relevance were emphasized in all of these studies. The evolving progress in poloxamers as adjuvants for viral delivery in clinical application holds great promise for improved gene-based treatments.

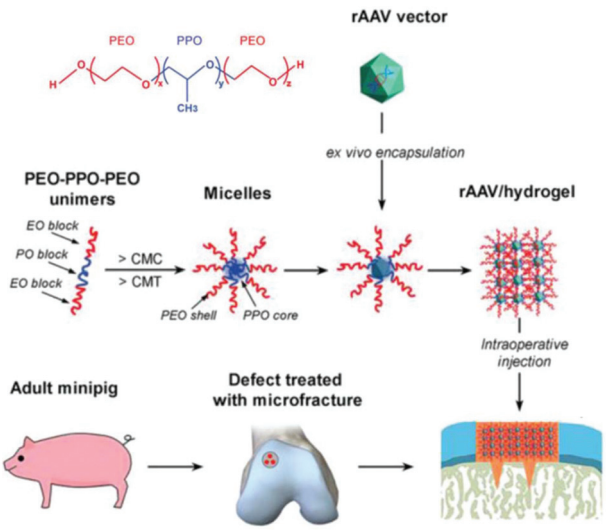

Fig. 6 Structure of poloxamer 407 and flowchart to generate rAAV poloxamer hydrogel systems for controlled release in knee implantations for minipigs. Adapted with permission from ref. 238. Creative Commons BY 4.0. 2019 John Wiley & Sons.

Dendrimers. Dendrimers are branched, radially symmetric molecules with a high density of functional surface moieties,

monodisperse size and internal cavities, which makes them an attractive nanomaterial as a vector carrier.[246] A general overview of various dendrimers used in non-viral gene delivery can be found elsewhere.[247]

Early works focused on inhibiting virus infections[248] or were used to control assembly[249] of viruses without any relation to gene delivery.

Polyamidoamine (PAMAM) is a common cationic dendrimer frequently used in non-viral gene delivery[250] and was applied for the first time to enhance TE of Ad by Vetter *et al.*[140] An epidermal growth factor receptor (EGFR) targeting peptide sequence was coupled to the dendrimer *via* a PEG-linker and coated on Ad, which resulted in increased specific infection to EGFR overexpressing tumor cells. The PAMAM complexed Ad showed lower cellular toxicity and higher TE than branched PEI.[140] EGFR targeting antibodies attached to PEGylated PAMAM also showed enhanced TE of Ad and tumor suppression *in vivo*.[251]

Further, polyphenylene dendrimers (PPD) were applied for viral delivery. PPD have amphiphilic properties forming a rigid globular architecture in solution with functional end groups organized like a shell in the periphery.[252] These features make PPD a promising protein-mimicking drug delivery agent.[253] Recently, Wu *et al.* reported PPD3 with engineered amphiphilic surface patches mimicking a protein corona, which enabled non-electrostatic interactions with Ad. The capsid proteins were complexed by PPD due to amphiphilic interactions, which resulted in enhanced TE to CAR negative cells and protection from neutralizing antibodies and the coagulation factor X.[116] Further remodelling of PPD3 led to one amphiphilic dendron branch, which was one quarter of the original PPD3 size (Fig. 7). This minimized dendron branch allowed Ad binding and non-covalent post-modification of viral capsids while maintaining advantageous properties of PPD3.[254] One disadvantage in using dendrimers is their laborious synthesis. Branched polymers carry a large number of functional groups, similar to dendrimers, but are often easier to synthesize. One example is the cationic copolymer EGDE 3,3′ containing the monomer ethylene glycol diglycidyl ether (EGDE) and 3,3′-diamino-*N*-methyl dipropylamine (3,3′), which induced higher TE of Ad to bladder cancer cells compared to the transfection reagent PEI.[255]

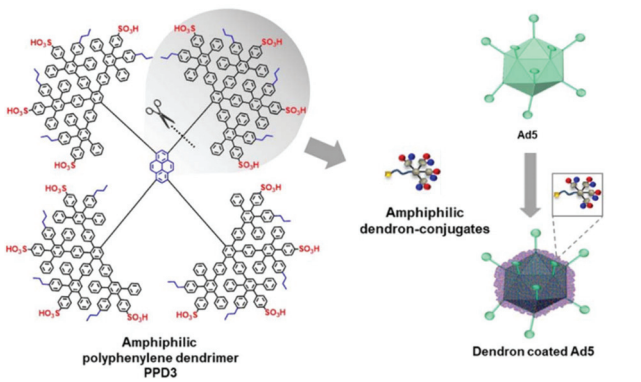

Fig. 7 Structure of amphiphilic polyphenylene dendron derived by desymmetrization of PPD3 dendrimer. Schematic chart shows dendron coated Ad5. Adapted with permission from ref. 254. Creative Commons BY 4.0. 2020 John Wiley & Sons.

Polysaccharides. Biomaterials from polysaccharides provide a versatile and biocompatible tool for clinical applications. Most polysaccharides used for viral delivery are hydrogels building a matrix for spatiotemporal release of VVs. Matrix-mediated viral delivery was recently reviewed elsewhere.[256] The following sections mainly cover saccharides in hydrogel applications.

Alginate is a linear copolymer consisting of β-D-mannuronate and α-L-guluronate and is commonly used as a hydrogel in food additives or pharmaceutical applications. Even though unmodified alginate hydrogels cannot interact specifically with mammalian cells,[257] a lot of progress has been made using such gels in controlled VV release in recent years. Ad encapsulated in alginate circumvent immune response *in vivo*[258] and augment long-term oncolytic Ad infection to tumor cells.[259] Distinct hydrogel-rAAV capsules were formed by tuning the composition and cross-linking temperature for alginate and poloxamer 407 containing systems. All of these rAAV capsules showed enhanced targeted delivery to hMSCs.[122] The spatiotemporal release kinetics of VVs can be stunted or enhanced by hydrogel physiochemical properties.[260,261] For example fabrication methods determine hydrogel mesh size, matrix affinity interactions and degradability and thus result in varying release kinetics for different sizes of VVs. LV delivery from $CaCO_3$ or $CaCl_2$ gelated alginate microcapsules generated by a microfluidic technique correlated with mechanical properties like hydrogel network mesh size and degradation rate and was controllable by the gelation method.[261] Moreover, low molecular weight alginate gels show faster degradation and release of LV, compared to more sustained release from high molecular weight alginate.[260] On-chip fabrication of alginate microgels gave access to blends of adjustable alginate formulations in a bottom up approach (Fig. 8A). Depending on the composition of different alginate molecular weights the degradation and release time of LV could be controlled *in vivo*.[262] Building on these results, Madrigal *et al.* reported that physical properties of various formulated alginates impact AAV and LV delivery to a different extent (Fig. 8B). LV release was tuned by initial strength and degradation rate of alginate gel, whereas AAV delivery remained unchanged independent of formulation. This result was interpreted as release of AAV by diffusive transport, whereas LV release is mainly controlled by degradation rate of alginate, which may be due to mesh/VP size relation. Consequently fast degradable alginate gels lead to higher TE for both AAV and LV.[263]

Mannose receptors were highly expressed in liver tissue and endocytosis-mediated cell entry is enhanced in the presence of mannose.[264] In order to target hepatocellular carcinoma, polymannose was covalently attached to Ad surface either by reductive amination[265] or by oxidation with sodium periodate.[266] The polymannose–Ad conjugate showed enhanced gene delivery to hepatocellular carcinoma cells both *in vitro* and *in vivo*.[266]

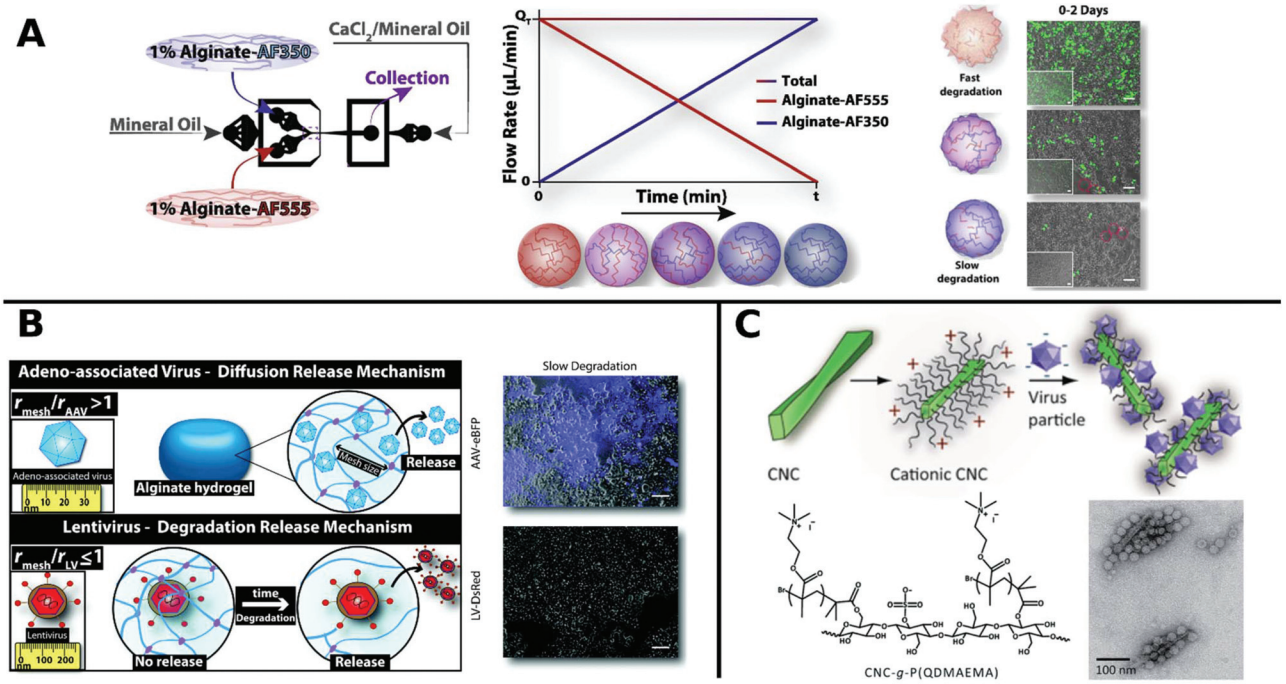

Fig. 8 A. Generation of gradient alginate microgel suspension *via* microfluidic blending. Gradual replacement of degradable alginate-AF555 formulation with non-degradable alginate AF350 leads to microgel composites with controllable degradation and LV release rates as observed in promoted GFP expression in HEK-293T cells by merged phase-contrast/fluorescent photomicrographs (scale bar: 100 μm). Adapted with permission from ref. 262. Copyright 2018 Elsevier. B. Illustration depicting the diffusion controlled release of AAV and degradation limited release of LV from alginate hydrogels. Merged phase-contrast/fluorescent images show gen-expression achieved after first day for AAV and LV in slow degradable alginate hydrogels (scale bar: 100 μm). Adapted with permission from ref. 263. Copyright 2019 The Royal Society of Chemistry. C. Schematic diagram shows the formation of VPs complexed with colloidal cellulose nanocrystals (CNC). TEM micrographs show CCMV complexed with CNC-*g*-P(QDMAEMA) (scale bar: 100 nm). Adapted with permission from ref. 274. Copyright 2014 The Royal Society of Chemistry.

Chitosan is a non-toxic, biodegradable cationic polymer, that shows neuroprotective effects after spinal cord injury by sealing nerve cell membranes[267] and promotes peripheral nerve regeneration.[268] Cross-linked with β-glycerol phosphate, chitosan yields compact fibrous hydrogels with increased charge density and binding to anionic particles as well as long-term gene expression over a period of seven days of encapsulated LV to dorsal root ganglia neurons.[127] Furthermore, chitosan has been used to replace the function of the viral envelope in non-infectious murine leukemia virus (MLV), which led to increased infectivity and transduction.[269] Hyaluric acid (HA) is a highly abundant linear polysaccharide in the extracellular matrix. HA applied as an injectable *in situ* forming scaffold can generate macroporous structures which are attractive vehicles for localized long-term release of viral particles. In a recent report, HA scaffolds of various pore sizes for LV delivery in mice mammary fat were compared with each other. Void spaces in HA scaffolds were created by different fabrication techniques. Nanoporous structures were achieved by cross-linking HA particles with PEG precursors, while macroporous architectures were created through *in situ* assembly of HA particles with PEG particles or enzymatic degradation of included PEG particles. Open, macroporous HA–PEG hydrogels displayed increased host cell infiltration and yielded higher TE compared to nanoporous hydrogels.[162]

Cyclodextrins (CD) are composed of 6–8 (α–γ) cyclic arranged glucose subunits forming a hydrophobic interior and a hydrophilic exterior toroid-shaped oligosaccharide, which is commonly used in pharmaceutical applications for drug delivery.[270] CDs have been applied as hydrogels in combination with other polymers[138,271] or as supramolecular linkers[272] for gene delivery as recently reviewed elsewhere.[273] Examples include Ad delivery to tumor microenvironment with a responsive polymer design containing PEG, PEI, MMP-sensitive peptide and β-CD.[138] Further, polypseudorotaxane gels based on either HA or chondroitin sulfate combined with α-CD were used to encapsulate and release rAAV to hMSCs. α-CD enhanced the viscoelasticity and storage modulus of the gels at physiological temperature and prolonged the permanence at the application site.[271] CD were also covalently attached to TMV surface in a supramolecular strategy to enable facile host–guest interactions with adamantyl moieties of imaging agents or chemotherapeutic drugs.[272]

In order to overcome anionic surface charges of some polysaccharides, modifications and combination approaches with other polymers have been made. For example, cellulose nanocrystals were surface-modified by atom-transfer radical polymerization with poly(*N*,*N*-dimethylaminoethyl methacrylate) to yield a brush-like cationic polymer (Fig. 8C). This polymer showed high-affinity virus binding.[274]

Bioreducible, branched polyaminoglycosides involving the antibiotic aminoglycoside tobramycin were proposed by Xu *et al.* as a transfection reagent.[275] Recently, they reported, LV–polyaminoglycoside complexes, which efficiently induce cell apoptosis to glioma cells by facilitating cellular uptake *via* endocytosis pathways.[276]

Polysaccharides are highly suitable biopolymers for viral gene delivery, mainly as hydrogels that adapt to the tissue environment. Release of VPs is achieved by degradation into non-toxic components. Combination of polysaccharides with polymers and functional molecules can further expand the range of material properties and influence controllable and efficient delivery of VPs in future applications.

Miscellaneous. In recent years, several additional polymers have been explored as VV delivery agents. In this section, miscellaneous emerging polymeric delivery systems are briefly introduced.

Several examples of hydrophilic polymers with similar stealth properties to PEG have been reported. For example, poly(2-oxazoline)s are thermoresponsive, hydrophilic polymers which gained increasing attraction for biomedical applications.[190,277] Recently, hepatitis B-like viral particles grafted with poly(2-ethyl-2-oxazoline)s were reported to reduce antigenic reaction.[278]

Similarly, poly(*N*-(2-hydroxypropyl)methacrylamide) (pHPMA) was conjugated through lysine residues on Ad surface and was able to shield from neutralizing antibodies, enhanced plasma circulation, decreased hepatotoxicity and indirect accumulation in tumor site. However, coating with pHPMA prevented efficient cellular uptake and reduced TE.[156,158,279] Francini *et al.* applied a new conjugation strategy with pHPMA bearing diazonium reactive groups, which can be bioconjugated onto a variety of functional amino acid residues of oncolytic Ad resulting in more efficient coupling and dense coverage of surface. While immunogenicity of conjugated VPs is low, TE is severely reduced due to low cellular uptake and delayed unpackaging of the vector. This study illustrates the necessary balance between efficient screening of the virus from being recognized by the immune system and sufficient cellular uptake in order to achieve effective transduction.[280] To take advantage of benefits from different systems, hybrids consisting of polymers and peptides have been investigated for enhancing TE. For example, arginine grafted onto poly(disulfide amine) were able to enhance TE, while being bio reducible and less immunogenic, when coated onto Ad.[114,281] AAV in hydrogels from PEG incorporated with poly-L-histidine showed ratio-controlled and pH-dependant swelling and TE (Fig. 9A).[282] PEG diacrylate matrices (PEGDA) blended with PLL can improve long-term, localized and efficient LV transduction when implanted *in vivo*.[283] Further, Kwon *et al.* introduced elaborate viral/non-viral chimeric systems by siRNA or DNA encapsulating polyketals assembling in a core–shell structure around AAV. This design enabled simultaneous gene transduction in a stimuli-responsive fashion by using of polyketals that are degraded in the acidic endosomal environment. Chimeric systems are promising platforms for obtaining syner-

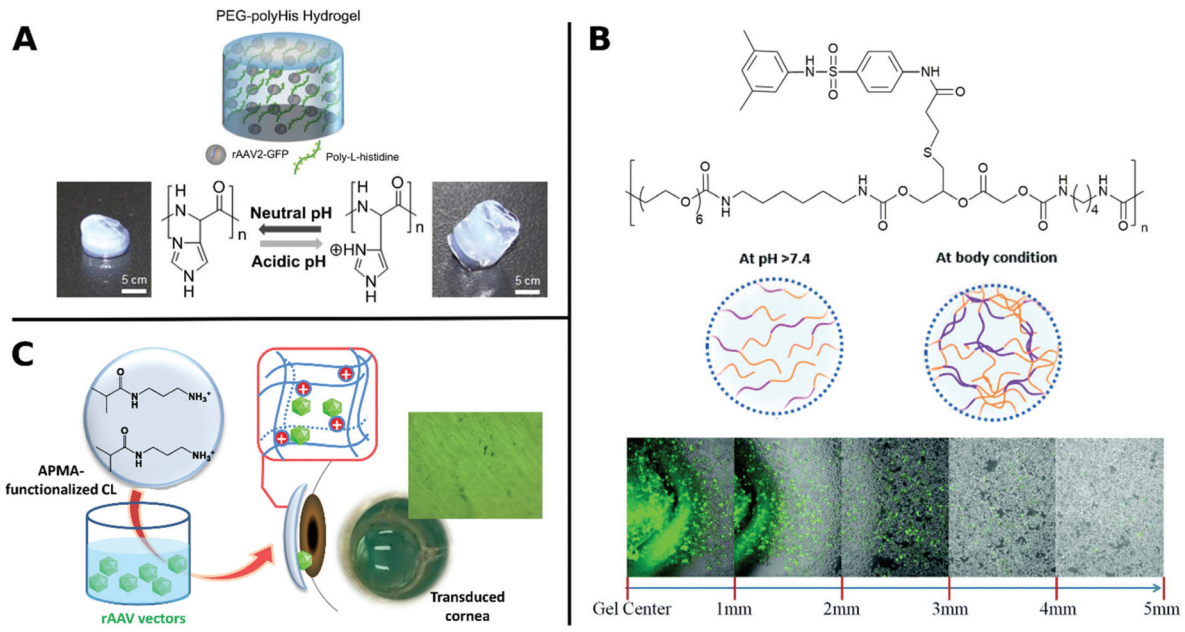

Fig. 9 A. Schematic illustration of rAAV embedded into PEG-polyHis hydrogel incubated under neutral (pH 7.4) or acidic (pH 6.0) conditions. Protonation of amine groups in polyHis under acidic conditions result in increased water uptake and swelling of the hydrogel. Adapted with permission from ref. 282. Copyright 2012 Elsevier. B. Structure of PUSMA and schematic illustration of sol–gel phase transition state at physiological conditions. Merged optical/fluorescence image of distance-dependent release of GFP-expressing Ad from PUSMA hydrogels. Adapted with permission from ref. 163. Copyright 2019 The Royal Society of Chemistry. C. Illustration of functionalization of APMA-hydrogel contact lenses for sustained VV delivery to cornea and picture of X-Gal stained bovine cornea after seven days in direct contact with rAAV encapsulated hydrogels. Adapted with permission from ref. 291. Creative Commons BY 4.0. 2020 MDPI.

gistic therapeutic effects by simultaneous expression and silencing of multiple genes. In cancer therapy, for example, simultaneous upregulation of pro-apoptotic mediators by AAV delivery and silencing of pro-survival genes by siRNA, can result in significantly more effective treatment.[284,285] Beside classical polymer systems, DNA-aptamers have also been utilized for targeted gene delivery. DNA-aptamers were covalently attached to viral capsid and selectively targeted Jurkat T cells and delivered cargo through an endocytic pathway.[286] DNA aptamers can further improve biocompatibility of viruses by shielding them from neutralizing antibodies and enhancing *in vivo* circulation rate.[287] Reducible disulfide linkages were utilized to covalently attach AAV to multiple DNA-aptamers, which were cleaved by intracellular glutathione and facilitate release of AAV in the cell, thereby enhancing TE.[288]

Polymers can also be used as 2D coatings to promote transduction by colocalization of viruses with host cells. So-called substrate mediated delivery was enabled *via* AAV capturing adhesive catecholamine surfaces (Fig. 10A).[289] Adhesive polydopamine-coated substrates were further improved in an simple upside down arrangement to adhere inverted quasi spherical droplets containing human neural stem cells (hNSCs) and AAVs (Fig. 10B). TE was enhanced by shortened path lengths and increased contact frequencies between cells and vectors due to the large contact angle of droplets on polydopamine coated surfaces.[290]

Several hydrophilic polymers have been applied as 3D materials, namely hydrogels, often for slow release of VPs. The biodegradable multiblock sulfamethazine and PEG-containing polyurethane (PUSMA) exhibit pH and thermoresponsive behavior and was used as an injectable hydrogel for *in vivo* oncolytic Ad delivery. The sol–gel transition occurred below pH 8.0 through a hydrophilic to hydrophobic transition leading to the formation of a porous hydrogel network. This hydrogel enabled spatiotemporal Ad delivery to injection site under physiological conditions (Fig. 9B).[163] An interesting example for the rAAV delivery to the cornea used hydrogels from a copolymer of hydroxyethyl methacrylate (HEMA) and aminopropyl methacrylamide (APMA) which can be worn as contact lenses (Fig. 9C). This transparent hydrogel network allowed high vector loading and controlled, long-term gene expression over a period of 14 days while being able to correct refractive errors.[291]

Microgels from vinyl ether acrylate-functionalized poly(vinyl alcohol) (PVA–VEA) and thiolated PVA–VEA were fabricated by a microfluidic technology and Michael-type cross-linking reaction to yield pH-degradable injectable spheres for efficient Ad delivery to tumor sites. The antitumor treatment could be reinforced by the addition of the bromodomain inhibitor JQ1. Accumulation of oncolytic Ad at the tumor site was enabled by pH-dependent controlled release from microgels. This combination approach paves the way for treatment of tumors by synergistic viral, chemo and immunotherapy as a promising system in clinical applications.[292]

Polystyrene coated with methyl methacrylate and divinylbenzene were formed into nanocups <500 nm by a template and applied as cavitation mediated carrier for oncolytic VV. Physical stimuli, *e.g.* ultrasound after intravenous injection resulted in enhanced transport and antitumor activity to treatment site.[293]

Several new polymeric systems and composites are emerging. Careful choice of the polymer and conjugation strategy allows the user to tailor pharmaceutical properties such as circulation time, controlled release of viral cargo and targeting of cells or tissues for next generation gene therapy.

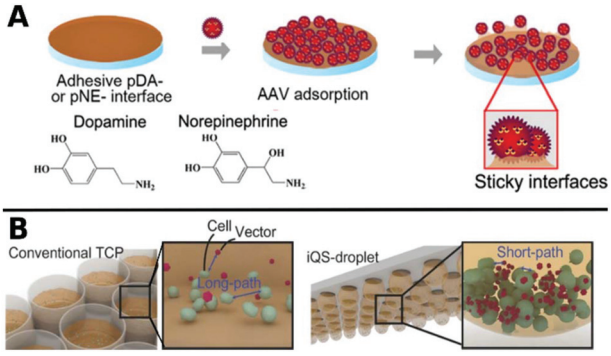

Fig. 10 A. Schematic illustrations of adsorption of AAV on catecholamine coated substrates. Adapted with permission from ref. 289. Copyright 2014 American Chemical Society. B. Schematic illustration demonstrating the promotion of cell–AAV interactions in an inverted quasi-spherical (iQS) droplet on polydopamine coated substrates due to shorter pathways compared to conventional tissue culture plates (TCPs). Adapted with permission from ref. 290. Copyright 2017 John Wiley & Sons.

Peptides

Due to their natural abundance and their ability for interactions with cells and viruses, bioactive peptides are highly interesting auxiliary agents for VV delivery.[296] Tailor-made peptides can be easily produced in large scale, are biocompatible and biodegradable.

Peptides applied for non-viral gene delivery were summarized recently elsewhere.[297] When used as enhancers for viral gene delivery three different classes of peptides have been applied so far: cell penetrating peptides (CPP), fibrils formed from self-assembling peptides and proteins.

The formation of peptide–virion complexes is typically achieved either by attractive electrostatic interactions between negatively charged viral particles and positively charged peptides or by bioconjugation of the respective peptide to the capsid. With the large structural variety offered by peptide sequences, it is not surprising that many different types of peptides have been reported as promoting viral gene delivery. Table 4 provides an overview of the reported peptides, their sequences, and physicochemical properties. Furthermore, the types of VVs that have been reported in combination with these peptides are highlighted.

CPPs. Cell penetrating peptides (CPPs), also called protein transduction domains (PTD), typically contain between 5 and 30 amino acids, have an overall positive charge and can facili-

Table 4 Overview of peptide sequences, properties and VP-peptide fabrication methods for the presented CPPs, fibrils and proteins

Name	Structure	Properties	Fabrication of VP-peptide	Ref.
$Tat_{47–57}$	YGRKKRRQRRR	h., c.	Inc. (Ad)/cov. conj. (Ad)/bioengineering (Ad)	311,315,318
$Tat_{48–57}$	GRKKRRQRRR	h., c.	Cov. conj. (Ad)	309
$Tat_{48–60}$	GRKKRRQRRRPPQ	h., c.	Cov. conj. (Ad)/bioengineering (Ad)	310, 316, 320 and 137
Tat HA2	CRRRQRRKKRGGDIMGEWGNEIFGAIAGFLG	Amph., c.	Bioengineering (AAV)/inc. (AAV)	322,328
OM-pBAEs	CRRR-PEG-CRRR	h., c.	Inc. (Ad)	312
R5	RRRRR	h., c.	Cov. conj. (Ad)	319
R8	RRRRRRRR	h., c.	Cov. conj. (Ad5/Ad/TYMV)/inc. (Pol., Ad)	311,316–318,320,329
R9	RRRRRRRRR	h., c.	Cov. conj. (Ad)/—	309 and 327
HP4	RRRRPRRTTRRRR	h., c.	Inc. (Ad)	119
K7	KKKKKKK	h., c.	Bioengineering (Ad)/—	137, 365, 366 and 326
Pep1	KETWWETWWTEWSQPKKKRKV	Amph., c.	Cov. conj. (Ad)/inc. (Ad)	311,318,320
$Pen/Antp_{62–77}$	RQIKIWFQNRRMKWKKGG	Amph., c.	Cov. conj. (Ad)/inc. (Ad)	308,309,311,318,320,328
Pro	VRLPPPVRLPPPVRLPPP	Amph., c.	Cov. conj. (Ad5)	316 and 317
n.n	NRPDSAQFWLHHGGGSLLGRMKGA	c.	Cov. conj. (hepatitis B)	314
12.51	TARGEHKEEELI	c.	Bioengineering (Ad)	313
THR	THRPPMWSPVWP	Amph., c.	Inc. (AAV)	321
KH27K	KHHHHHHHHHHHHHHHHHHHHHHHHHHHK	h., c.	Inc. (polyomavirus)	329
FUSO	CGLFEALLELLESLWELLLEA	a.	Inc. (polyomavirus)	329
LAH4	KKALLALALHHLAHLALHLALALKKA	Amph., c.	Inc. (AAV2 and AAV8, Pol)/inc. (LV)	328,329,351
Vectofusin-1	KKALLHAALAHLLALAHHLLALLKKA	Amph., α-helix	Inc. (LV)	130 and 354
$PAP_{248–286}$	GIHKQKEKSRLQGGVLVNEILNHMKRATQIPSYKKLIMY	Amyloid, c.	Inc. (HIV-1)	336
$PAP_{85–120}$	IRKRYRKFLNESYKHEQVYIRSTDVDRTLMSAMTNL	Amyloid, c.	Inc. (HIV-1)	337
$SEM1_{45–107}$	GQHYSGQKGKQQTESKGSFSIQYTYHVDANDHDQSRKSQ-QYDLNALHKTTKSQRHLGGSQQLL	Amyloid, c.	Inc. (HIV-1)	338
EF-C	QCKIKQIINMWQ	Amph., c. amyloid	Inc. (RV, LV)	118
EP2	QCKIKQIINMWQEVG	Amyloid, c.	Inc. (HIV-1)	343
EP3	NITLQCKIKQIINMWQEVG	Amyloid, c.	Inc. (HIV-1)	344
P13	Ac-NWFDITNWLWYIK-NH_2	Amyloid, c.	Inc. (HIV-1)	339
P16	Ac-NWFDITNWLWYIKKKK-NH_2	Amyloid, c.	Inc. (HIV-1)	339
P16-D	Ac-NWFAITNWLWYIKKKK-NH_2	Amyloid, c.	Inc. (HIV-1)	341
CD4bs-M	XGXSGGDPEIVTXKXXLTRDGGN (X = ε-aminohexanoic acid)	Amyloid, c.	Inc. (HIV-1)	346
Fmoc-SAP	Fmoc-DDIKVAVK	Gel, c.	Coinjection (LV)	348
DPF1	INMWQG	Fibrils	Inc. (HIV-1)	342
RAD16-1	Ac-RADARADARADARADA-CONH2	Fibrils	Cogelation (AAV)	234
KK, KY	KYKGAIIGNIK, KYRSGAITIGY	Amyloid		347
α-Syn	140 amino acids, consecutive KTKEGV	Amyloid	Inc. (RV)	117
Protamine sulfate	Mixture of polypeptides	c.	Inc. (RV, Ad, LV)	243, 356 and 357
Retronectin	574 amino acids, 63 kDa	c.	Inc. on precoated substrates (LV, RV)	359 and 362–364
PEG–PLL	PEG_{12000}–PLL_{48}/20 kDa PEG dendrimer–PLL	c.	Inc. (RV)/inc. (LV)	368 and 369
PLL	Poly-L-lysine 150–300 kDa	c.	Inc. (VLP)	367
Fibrin	Fibrinogen and thrombin mixture	Fibrous gel	Cogelation (LV, Ad/AAV)	372, 374, 235 and 373
Collagen	Collagen type 1	Fibrous gel	Cogelation (LV)	126
	Collagen/hydroxyapatite ($Ca_5(PO_4)_3(OH)$)	Gel	Cogelation (LV)	126
Gelatin	Hydrolyzed collagen	Gel	Cov. conj: (Ad)/cogelation (Ad)	385 and 386
Serum proteins	HSA, LDL, Transferrin	c.	Inc. (AAV8/HIV-1)	375, 377 and 376
SELP	GAGAGS and GVGVP blocks	Gel	Inc. (Ad/GLV-1h68)	382, 383 and 384

Abbreviations: cationic (c.), hydrophilic (h.), amphiphilic (amph.), anionic (a.), incubation (inc.), covalent conjugation (cov. conj.), adenovirus (Ad), adeno-associated virus (AAV), lentivirus (LV), retrovirus (RV), tobacco mosaic virus (TMV), polyomavirus (Pol).

tate efficient entry into cells. CPPs can exhibit various secondary structures from random coil to α-helix,[298] and typically have a large amount of basic amino acids like arginine or lysine and have an amphiphilic or hydrophilic character. CPP motifs are widespread in Nature, *e.g.* in heparin-, RNA- and DNA-binding proteins, signalling peptides or viral proteins and allow receptor independent cell-entry.[299] Since the discovery of the first CPP derived from HIV-1 binding protein Tat in 1988,[300,301] more than 1000 CPPs predominantly from natural origin or found by phage display are known today.[302]

Their ability to transport variable cargo across cellular membranes has made CPPs a facile tool for the delivery of DNA and RNA, liposomes, nanoparticles, proteins, and drugs as reviewed elsewhere[303,304] and of viral nanoparticles for efficient transduction as comprehensively reviewed.[305,306] In this segment, we highlight the most important developments in CPPs for facilitating transport of VP into target cells.

The most straightforward and easy way to obtain CPP–virus complexes is by co-incubation, making use of electrostatic interactions between positively charged CPPs and negatively charged VP. This method can be applied independently of the virus type, and ratio of CPP to VP can be easily adjusted in solution. However, the formation of CPP–VP complexes cannot be controlled, there is batch-to-batch variability and the highly positively charged CPP–VP complexes are prone to aggregation in physiologic conditions, for example, in the presence of electrolytes or serum (Fig. 11).[307] A more stable but demanding approach is to covalently connect CPPs to the vector capsid. Early studies have demonstrated both approaches: the utilization of electrostatically bound Pen and Ad complexes to facilitate viral gene transfer in muscle cells[308] and covalently bound Tat–Ad conjugates for delivery to tumor cell lines.[309] By covalently attaching Tat to exposed lysine residues of the vector capsid *via* an MHS linker, transduction efficiency was further improved.[310] Similar observations were made for Tat, Pen, R8 and Pep1 when they were covalently attached to PEGylated adenoviruses (Fig. 11).[311]

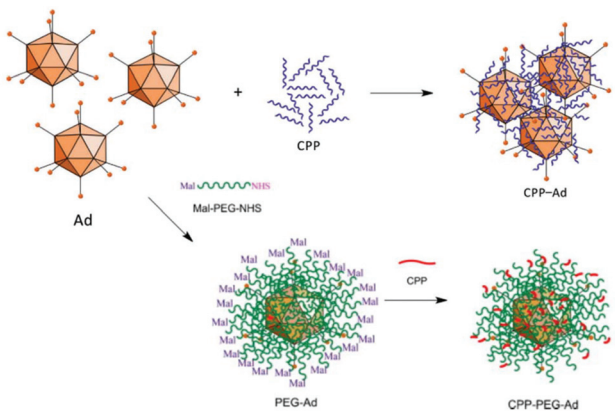

Fig. 11 Approaches for producing CPP–Ad complexes (top) and conjugates (bottom). Complex formation results from electrostatic interactions between positively charged CPPs and negatively charged VPs. Covalent conjugations of CPPs are conducted on viral capsids, *e.g. via* bifunctional PEG linkers. Adapted with permission from ref. 307 and 311. Copyright 2013 and 2015 Elsevier.

Coating of CRRR-*co*-PEG-*co*-CRRR onto an oncolytic Ad (Fig. 12A) enhanced transduction in tumor sites with a longer blood circulation time and lower liver sequestration was achieved.[312]

In addition to CPPs derived from natural peptides, screening for suitable sequences by phage display has become a powerful method to discover new CPPs for enhanced transduction and targeted virus delivery.[313,314]

It was further possible to broaden the tropism of Ad by gene transfer to otherwise non-transducable CAR-negative cells. To this end, the surface knobs were modulated with Tat peptides,[137,315] Tat peptides were attached to surface bound lysine residues[316,317] or simply incubated in solution.[318] Other examples for hard-to-transduce cell types that are successfully targeted with CPPs include Ad delivery to resistant stem cells and various cancer cells which was efficiently achieved by addition of arginine-rich HP4 derived from herring protamine.[119] Furthermore, by decorating CPPs onto capsids *via* hydrazone chemistry, plant viruses like CPMV and TYMV were able to transduce otherwise non-infectable mammalian cells.[319,320]

Specific CPPs capable of crossing the blood brain barrier (BBB), can facilitate the delivery of CPP virus-complexes to the central nervous system after systemic application (Fig. 12B).[321] In an interesting approach AAV containing a brain derived neurotrophic factor fused with Tat were delivered intranasally to the central nervous system to act as an antidepressant in mice.[322]

Frequently used CPPs for VV delivery are various Tat peptide fragments, oligoarginines, and penetratin (Pen). These peptides show different transduction enhancing properties depending on the viral particle, host cell, and CPP concentration.[318] This difference in enhancement is believed to result from structural properties of the CPPs as well as their respective cellular entry mechanism. In general, various endocytic and non-endocytic cell entry pathways are controversially discussed in the literature.[323,324]

Regarding the structure, one requirement for the electrostatic stabilization of the virus peptide complex, as well as adhering to and crossing the negatively charged lipid bilayer of cells, is a high amount of positive charges in the peptide.[318] Among peptides with positive charge those rich in arginine showed greater membrane permeability than CPPs with high amount of other cationic amino acids like lysine, histidine or ornithine.[325,326] Beside the charge, hydrophilicity can also influence interaction with the membrane. It is believed that the higher performance of hydrophilic peptides like Tat and oligoarginines is due to stronger interactions with heparan sulfate proteoglycans of the cell membrane, whereas amphipathic peptides like Pep1 interfere with electrostatic binding of proteoglycans, thus resulting in lower transduction efficiency.[318] Interestingly, a higher CPP concentration for VV delivery did not necessarily lead to a linear increase in transduction efficiency as shown for Tat, Pen, oligoarginine and

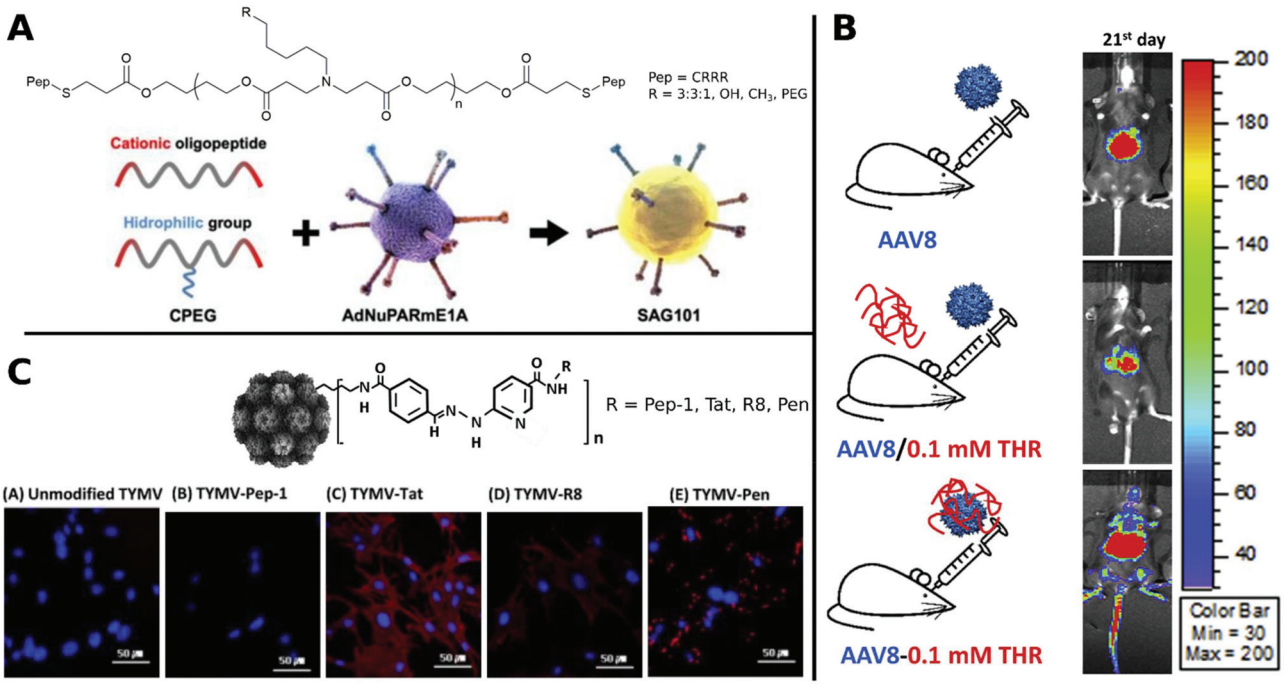

Fig. 12 Selection of CPPs. A. Schematic representation of oncolytic Ad coating with oligopeptides. Adapted with permission from ref. 312. Creative Commons BY 4.0. 2020 Ivyspring International Publisher. B. Schematic representation of three groups of AAV8 administration: AAV8 only, AAV8/0.1 mM THR (not incubated) and AAV8–0.1 mM THR (incubated). Luminescence expression images were taken on 21st day after systemic administration. Adapted with permission from ref. 321. Copyright 2018 Elsevier. C. Hydrazone conjugated TYMV–peptide. Confocal analysis of transfected BHK21 cells stained with Hoechst 33258 dye (blue) with CPP modified and unmodified TYMV visualized with AF594 (red) (scale bar: 50 μm). Adapted with permission from ref. 320. Copyright 2018 Elsevier.

Pep1.[316,318] Chirality also significantly affects cell permeability. A 9-mer of poly-D-arginine showed five-fold higher cellular uptake than poly-L-arginine.[327]

Mechanistic pathways for the TE of AAV–CPP complexes were investigated by delivering AAV to non-permissive cell lines and blocking specific receptors. The tested peptides Pen, Tat-HA2 and LAH4 facilitate energy dependent and independent endocytosis as well as receptor-mediated pathways for the internalization of AAV.[328] Further, Tat–Ad promoted cellular uptake *via* heparane sulfate receptors on the membrane surface, while the oligoarginine adenovirus conjugate R8-Ad was more dependent on chondroitin sulfate B receptors. This lays the foundation for CPP-dependent virus delivery.[316]

In a comparative study, TYMV was bioconjugated to Tat, R8, Pen or Pep-1. Improved efficiency was observed for Tat, R8 and Pen, whereas Pep-1 showed no change in transduction. This was traced back to different internalization routes and distributions of CPP–virus complexes in the cytoplasm and visualized by confocal images (Fig. 12C).[320]

Recently, Váňová *et al.* reported further mechanistic explanations for different TEs by investigating the influence of various CPPs on the activity and stability of VPs. They found that KH27K, FUSO, R8 and LAH4 affected the stability of VPs in different ways. KH27K promotes the aggregation and enlargement of VPs, while LAH4 destabilizes VPs but still enhances infection by concentrating them onto the cell surfaces (Fig. 13A).[329] This study provides an example for enhanced TE *via* virus disassembly, which was suggested in an earlier report covering Pen, Tat and R8[311] and provides the basis for further explanations of observed differences between CPPs.[320]

Peptide fibrils. The intrinsic ability of some peptides to self-assemble into higher order structures allow for numerous applications as functional biomaterials.[330,331] In the last ten years, peptide fibrils caused a paradigm shift in viral gene delivery from using mostly spherical particles to anisotropic, fibrillar nanocarriers, due to their convenient handling and high TE.

In contrast to CPPs, fibrillar peptides typically have a distinct secondary structure and cannot necessarily cross cell membranes. Enhanced viral gene delivery is achieved by colocalization of electrostatically complexed peptides and viral particles with the cellular membrane. Due to the high aspect ratio and rigidity of fibrils it is assumed that they cannot adapt to and cover virus surfaces thoroughly, thus creating excess positive charges in the virus–fibril complex for electrostatic interaction with cell membranes.[332]

The most thoroughly investigated fibrils for viral gene delivery are amyloids. Amyloid fibrils are highly stable and rigid, which makes them interesting materials for applications where long-term stability or tolerance of harsh conditions are required. For a long time, amyloid-forming peptides have exclusively been associated with diseases like plaque formation in Alzheimer's disease.[333,334] However, these structures were also found in non-pathogenic contexts as functional amyloids,

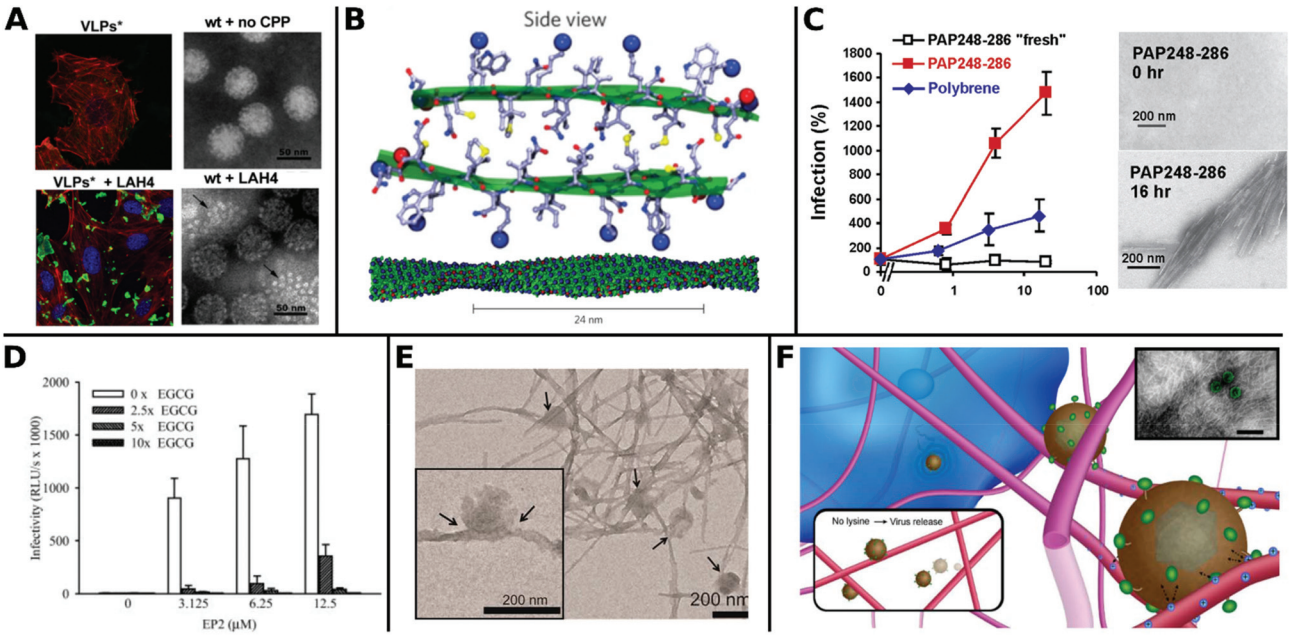

Fig. 13 Overview of fibril virus interaction and bioactivity. A. Left panels: Confocal analysis of 3T6 cells (actin = red, nucleus = blue) after association with MPyV virus like particles (green) without (upper image) and with LAH4 peptide (bottom image). Right panels: TEM images of virions in buffer were stable and compact (upper image), whereas coincubation with LAH4 led to virions disassembling into pentamers (bottom image) (scale bar: 50 μm). Adapted with permission from ref. 329. Copyright 2020 Elsevier. B. Molecular model of EF-C amyloid peptide fibril. Adapted with permission from ref. 118. Copyright 2013 Springer Nature. C. Overnight incubated PAP248–286 enhances HIV-1 infection of TZM-bl cells more efficiently than non-incubated fresh PAP248–286 or Polybrene. TEM images show structural change to peptides before and after incubation for 16 h (scale bar = 200 nm). Adapted with permission from ref. 345. Copyright 2007 Elsevier. D. Decrease of HIV-1 R5 infectivity after EGCG addition on EP2 peptides. Adapted with permission from ref. 344. Copyright 2014 John Wiley & Sons. E. TEM image of virions adhered to α-Syn fibrils (scale bar = 200 nm). Adapted with permission from ref. 117. Copyright 2018 American Chemical Society. F. Schematic representation of electrostatic interaction between LV and fibrillar peptide. Charge based immobilization of LV is achieved through peptide functionalization with additional Lysine to give Fmoc-DDIKVAVK. Inset shows TEM image of nanofibrous network with green colored LV (TEM scale bar = 200 nm). Adapted with permission from ref. 348. Copyright 2020 Springer Nature.

for example on bacteria surfaces[335] and in seminal fluids.[336] In the latter case, prostatic acidic phosphatase (PAP), a semen derived enhancer of virus infection (SEVI) with two distinct regions $PAP_{248–286}$ and $PAP_{85–120}$, and semenogelins (SEM) was found to form amyloidal structures and enhance HIV-1 infection.[336–338] These very first findings sparked further research in amyloid peptides for viral gene delivery.

Transduction enhancing peptides can also be found in virus envelopes. The optimization of the glycoprotein fragments $gp120_{417–428}$ (EF-C, commercialized as Protransduzin) and $gp41_{671–682}$ (P13 and P16) from the HIV-1 envelope and transmembrane protein, respectively, form small amyloid fragments that assemble into cationic nanofibrils of several hundred nanometers in length and a few nanometers in diameter (Fig. 13B). These short fragments showed higher TE compared to Tat, polybrene, DEAE and SEVI in various cell lines including difficult to transduce TZM-bl cells, while being cost effective and convenient in handling.[118,339] Fluorescent dyes coupled with free amino groups in EF-C have been introduced as a tool to study plasma stability and *in vivo* biodistribution while maintaining the structural and functional properties of non-labeled EF-C.[340] Point mutations in the peptide sequences had a great impact on TE as shown for P16-D, where a substitution of aspartic acid with alanine resulted in increased activity.[341] Sequence variations of $gp120_{417–428}$ yielded the 6-mer DPF1 and longer sequences EP2 and EP3 which accelerate amyloid formation of SEVI and SEM and enhanced TE.[342–344]

The fibrillar structure is necessary for enhanced VV delivery (Fig. 13C)[345] as shown by the following example: addition of epigallocatechin gallate, a main compound of green tea, which inhibits amyloid formation resulted in lower TE (Fig. 13D).[344] Amyloids can also help to broaden the tropism of viruses. For instance HIV-1 was able to transduce in hardly infectable CD4 negative cells with the addition of amyloidal CD4bs-M peptides.[346]

Moreover, the cofibrillation of amyloid α-Syn with positively charged polymers like poly-L-lysine or chitosan enhanced TE of amyloid fibrils as recently reported by Maji and coworkers. The authors suggested that these positively charged additives increased electrostatic interactions and local density of virions on cell surface, thereby facilitating the transduction (Fig. 13E).[117]

Besides using derivates of naturally occurring amyloids for vector delivery, amyloids can also be computationally designed as shown for KK and KY, which enhanced DNA delivery to

mammalian cells by forming DNA–amyloid complex and overcoming charge repulsion, as discussed for VVs.[347] Since synthetic amyloid fibrils have so far only been applied in *ex vivo* studies, the question of fibril stability and degradability *in vivo* remains open.

Hydrogels formed from fibrillar peptides are emerging as VV delivery scaffolds, due to their simple handling and spatiotemporal release of viruses. For instance, Fmoc-SAP derived from the epitope IKVAV enabled localized delivery of LV after implantation into the central nervous system of mice. The modification of the sequence with an additional lysine at the C-terminus increased electrostatic interactions and immobilized the VP to obtain focal gene delivery to the site of injection (Fig. 13F).[348] The peptide hydrogel RAD16-1 (commercialized as PuraMatrix™) is frequently used in cell culture. It displays favourable nano-structural and biomechanical properties and promotes the proliferation of various mammalian cells.[349] Rey-Rico *et al.* reported RAD16-1 hydrogels for durable genetic modifications to stem cells by enhanced localized AAV delivery over a period of 21 days *in vivo*.[234]

The LAH4 peptide family has attracted growing interest in recent years as a DNA transfection reagent as well as for VV delivery.[328,350,351] One commercialized derivative of the LAH4 family is Vectofusin-1.[351] This 26-mer cell penetrating peptide is the first transduction enhancer with α-helical fibrillar structure. Studies have shown enhanced TE of Vectofusin-1 compared to other delivery agents such as Tat, Pen, LAH4 derivatives, KH27K, R8, FUSO, polybrene, and retronectin.[328,351–355] Interestingly, a variation in the histidine sequence order or peptide length resulted in a significant change of bioactivity. A minimum length of 21 amino acids of the LAH4 peptide family was found to be necessary for successful vector delivery.[355]

Polypeptides. Polypeptides for VV delivery are mainly derived from natural sources and are typically used as scaffolds for enhanced delivery by colocalization.

Protamine sulfate is an early examples for viral transduction enhancers, originally derived from salmon sperm and clinically used for treatment of heparin overdose. This substance is actually a mixture of several polycationic peptides and facilitates virus–cell interactions by charge neutralization similar to other enhancers like polybrene, DEAE dextran or poly-L-lysine.[356,357] In comparative transduction studies of LV delivery to CD34+ stem cells, protamine sulfate showed a TE similar to Vectofusin-1 and higher than that of Retronectin.[243] TE could be enhanced even further by combining protamine sulfate with chondroitin sulfate.[358]

Retronectin is a 574 amino acid long recombinant polypeptide based on the fibronectin fragment CH296. It is one of the first commercially available enhancers for VV delivery and has already been tested in clinical trials.[359–361] A typical workflow for transduction involves the coating of Retronectin on a solid surface, the addition of viral particles and cells followed by spin centrifugation.[362] To overcome the limitations of the laborious preparation on surfaces and the necessity of centrifugation, various alternative approaches have been developed. The coating of epoxy-modified paramagnetic beads with Retronectin enables the effective capture of retroviral particles from solution and provides access to remote-controllable transduction.[363] *In vivo* LV delivery to stem cells *via* intra-bone marrow injection in mice showed enhanced TE when Retronectin was co-injected with the virus.[364]

Poly-L-lysine (PLL) is a popular additive in cell cultures for reinforcing adhesion of cells to culture dish. It is used as short oligomers up to polypeptides of several 100 kDa and can either be covalently attached to virus surface or simply coated on surfaces.[365–367] PLL has been investigated as a replacement for polybrene in viral delivery by creating the block copolymer PEG_{12000}–PLL_{48} which was coated on RV through electrostatic interactions. In contrast to polybrene, this copolymer augments RV transduction to delicate primary cultured brain cells without cytotoxic effects.[368] Further optimization of PEG–PLL compositions were made by functionalizing four-arm PEG acrylates with PLL of different molecular weights ranging from 1–70 kDa. Increased TE was observed for increased molecular weights of PLL.[369] Moreover, PLL has been applied as a mimic of the viral envelope and enhanced cellular internalization and transduction of virus-like particles.[367] Recently, modifications of PLL_{120} with *p*-toluylsulfonyl arginine resulted in change of conformation from random coil to α-helix, which gave higher DNA-transfection rates due to higher cell-permeability.[370] Layer-by-layer deposition – referred to as protein surface precipiation here – of PLL and dextran sulfate sodium salt were applied onto Ad surface to obtain nanospheres that protect Ad from antigenic reactions and thereby enhanced TE (Fig. 14A).[371] These recent findings highlight the importance of material deposition techniques and conformational structure for viral gene delivery.

Enhanced viral delivery with spatiotemporal control can be achieved by fibrous protein scaffolds, such as fibrin. For example, when VPs were incorporated in fibrin, a local, long-term release of Ad,[372] AAV[235,373] and LV[374] *in vitro* and *in vivo* was observed at the injection site up to 14 days. Fibrin scaffolds could be further improved by the addition of hydroxyapatite nanoparticles, resulting in stronger trapping as well as slower release of VPs from scaffolds[374] (Fig. 14B) and gels.[126]

Vupputuri *et al.* reported that protein impurities in cell culture can lead to increase or decrease of viral TE. By carefully adjusting the amount of protein added to viral particles, they could improve TE of polymer–virus complexes. Proteins bound to VPs can increase TE due to sedimentation of heathier particles, however at the same time cellular uptake pathway is changed by larger VPs thus protein addition has to be carefully balanced.[132] This observation have far-reaching consequences for comparability of studies concerning TE, when taking batch-to-batch variability of culture medium and VP purification into account. For example, proteins from the human serum can also augment AAV transduction. Cationic human serum albumin (cHSA), an abundant endogenous transporter protein in blood, increased transduction of an *in vivo* hemophilia B mouse model fivefold.[375] PEGylated cHSA improved TE of HIV-1 to TZM-bl cells while displaying lower immuno-

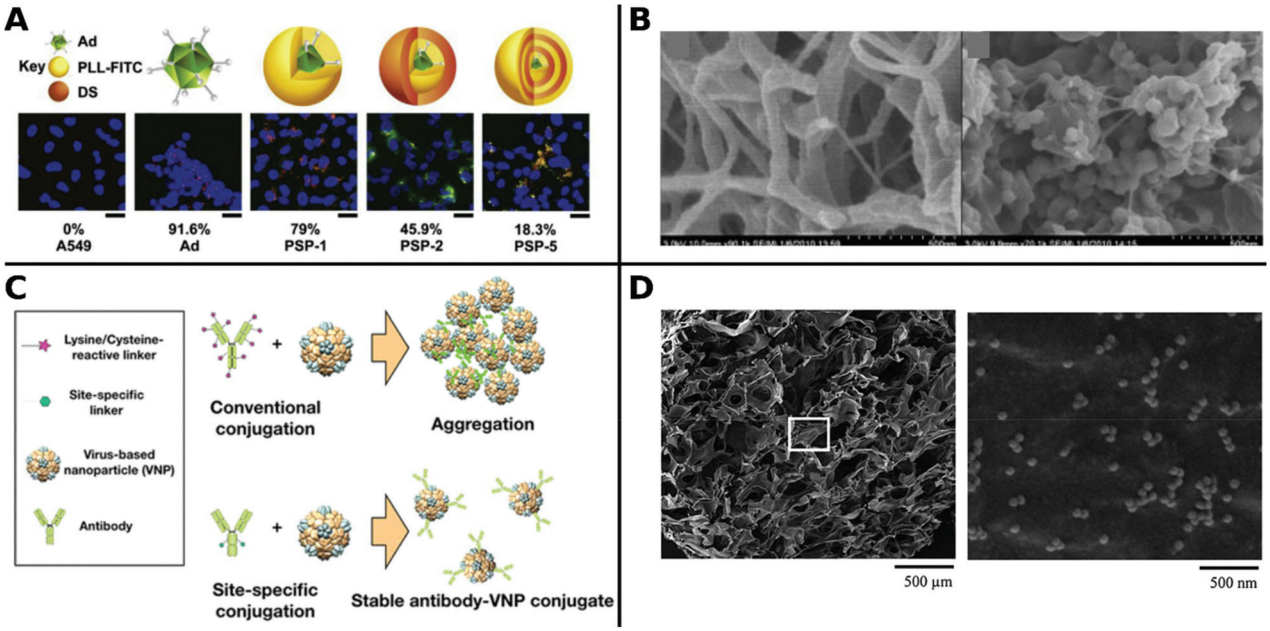

Fig. 14 A. Schematic representation of naked Ad and layer-by-layer PLL/dextran sulfate sodium nanosphere coated Ad (PSP). Fluorescence images show gene expression with percentual transduction rates (scale bar: 15 μm). Adapted with permission from ref. 371. Copyright 2019 Elsevier. B. SEM images of fibrin scaffold with LV incorporated without (left) and with hydroxylapatite (right) (scale bar: 500 nm). Adapted with permission from ref. 374. Copyright 2012 Elsevier. C. Schematic illustration of site-specific antibody conjugation on VP capsid surface. Adapted with permission from ref. 378. Copyright 2020 American Chemical Society. D. SEM images of Ad immobilized on gelatin sponges (scale bar: left 500 μm, right 500 nm). Adapted with permission from ref. 385. Copyright 2013 John Wiley & Sons.

genicity.[376] Combinations of HSA, low density lipoproteins (LDL) or transferrin yield AAV–serum protein complexes which enhanced transduction to liver *in vivo*, prevented binding of AAV to other proteins, and suppressed nonspecific binding to cells.[377] Finally, site-specific antibody conjugation to TYMV, CPMV and bacteriophage *via* azide–alkyne click chemistry was reported by Park *et al.* (Fig. 14C).[378]

These examples highlight that natural proteins can efficiently enhance viral delivery. However, concerns of potential immunogenicity of xenogeneic materials exist.[379] Nature-inspired, synthetic silk elastin-like protein polymers (SELPs) may circumvent this problem. SELPs mainly consist of repeating amino acid blocks based on the typical silk (GAGAGS) and mammalian elastin (GVGVP) motifs and were first reported for Ad release in cancer gene therapy in 2004.[380,381] SELPs showed enhanced, local, long-term Ad delivery up to four weeks in various studies.[382,383] In a comparative study with poloxamer 407, SELPs showed promising results with a superior TE, lower toxicity and immunoreactivity.[384] Furthermore, collagen, an abundant fibrous protein in the extracellular matrix, showed long-term localized gene expression *in vivo* up to 4 weeks when it was co-gelated with LV.[126] Organic–inorganic hybrid systems, such as collagen hydrogels combined with hydroxyapatite nanoparticles retain LV over a period of four weeks *in vivo* and increase local delivery efficiency even more.[126] Moreover, gelatin gels, which consist mainly of hydrolysed collagen, were also applied for viral delivery. Gelatin sponges and Ad were functionalized with biotin to link them after avidin addition. The virus tethered in a virus–biotin–avidin–biotin–gelatin arrangement showed enhanced spatiotemporal TE *in vivo* after implantation for bone regeneration (Fig. 14D).[385] Local long-term gene expression was also shown for oncolytic Ad delivery from gelatin gels *in vivo* up to 20 days. These gels could further protect Ad from immune response and prevent unwanted delivery to healthy sites.[386]

Lipids

Lipids are amphiphilic molecules typically consisting of a hydrophilic head group and a hydrophobic tail. Lipids are the main component of cellular membranes, virus envelopes and extracellular vesicles, which are Nature's endogenous nanocarriers to deliver biological information from cell to cell.[387] The unique ability of lipids in aqueous media to form bilayers in a vesicular structure (liposomes) and spherical monolayers (micelles) enables them to encapsulate both hydrophilic and hydrophobic cargo.[388,389] After encapsulation the cargo is protected from degradation as well as immune reaction[390] and can further address specific targets by functionalization at the liposome's outer sphere.[391] Since their first discovery in the 1960s,[392] liposomes have been extensively used as delivery agents in clinical trials and pharmaceutical industry.[393,394]

In non-viral gene delivery, lipids are currently the gold standard. For example, liposomes were applied as DNA and RNA nanocarriers[395,396] as well as mimics of the viral envelope for gene delivery.[397] Recent reviews on non-viral application of lipids for gene delivery can be found elsewhere.[398,399] Lipids

Table 5 Overview of lipids and lipid formulations, properties and fabrication methods

Name	Structure	Properties	Fabrication of VP lipid complexes	Ref.
Chol	Cholesterol	z., fuso.	Inc. (Ad)	405
DOTAP/Chol	(1,2-Dioleoyloxypropyl)-*N*,*N*,*N*-trimethylammonium chloride/cholesterol	c.	Lipid bilayer wrapping (Ad)/inc. in preformed liposomes (Ad)/self-assembly around VP (Ad)	409, 390, 53 and 416
DOTAP/DOPE	DOTAP/(1,2-dioleoyl-*sn-glycero*-3-phosphoethanolamine)	c./fuso., z.	Self-assembly around VP (Ad)	409
DOTAP/DOPE/Chol		c./fuso. z.	Inc. (dry film or extrusion method) or assembly around VP (RV)	410
DMPC/Chol	Dimyristoyl phosphatidylcholine/cholesterol	z.	Self-assembly around VP (Ad)	53 and 409
DMPC/Chol/apoA-1	DMPC/Chol/apolipoprotein A-1	z.	Inc. in preformed liposomes (Ad)	124
DMPC/Chol/DSPE-PEG		z.	Self-assembly around VP (Ad)	53
TMAG/DLPC/DOPE	*N*-(α-Trimethylammonio-acetyl)didodecyl-D-glutamate chloride/dilauroyl phosphatidylcholine/DOPE	z.	Inc. in preformed liposomes (AAV, rAd)	407 and 417
DC-Chol/DOPE	3 beta [*N*-(*N*′,*N*″-dimethylaminoethane)-carbamoyl]cholesterol/DOPE	z.	Inc. in preformed liposomes (RV, Ad + siRNA)	401 and 423
Lipofectamine (DOSPA/DOPE (3/1))	2,3-Dioleyloxy-*N*-[2(sperminecarboxamido)ethyl]-*N*,*N*-dimethyl-1-propaninium trifluoroacetate/DOPE	c., fuso.	Inc. in preformed lipid-DNA complex (AAV)/inc. in preformed liposomes (RV/HSV/Reovirus)	415, 400, 418 and 420
Lipofectin (DOTMA/DOPE (1/1))	*N*-[1-(2,3-Dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride/DOPE	c., fuso.	Inc. in preformed liposomes (RV)	400 and 414
Lecithin/Chol (4/1)		z., fuso.	Inc. in preformed liposomes (alphavirus)	421
Lysolecithin	L-α-Lysophosphatidylcholine	z., fuso	Preconditioning of host cells prior to LV injection	424–427
PC/PG/Chol	Phosphatidylcholine/phosphatidylglycerol/Chol	z.	Reverse-evaporation unilamellar vesicle (MLV)	406
PEGDE	[1,2-Distearoyl-*sn-glycero*-3-phosphoethanolamine-*N*-[methoxy(poly-ethylene glycol)-2000]		Inc. in preformed liposomes (Ad)	432 and 433
DSPE-PEG-biotin	(1,2-Distearoyl-*sn-glycero*-3-phosphoethanolamine-*N*-[biotinyl(polyethylene glycol)2000])		Inc. in preformed liposomes (RV)	435
PC/CHEMS/Chol-PEG-MMP-peptide	PC/CHEMS/Chol-PEG2000-GPLGIAGQC	a., MMP responsive	Inc. in preformed liposomes with Ca^{2+} ions (Ad)	413
DOPE/CHEMS (3/2)	DOPE/cholesteryl hemisuccinate	a., fuso	Inc. in preformed liposomes with Ca^{2+} ions (Ad)	412
CHEMS/PC/Chol (4/5/1)	CHEMS/phosphatidylcholine (PC)/Chol	a.	Inc. in preformed liposomes with Ca^{2+} ions (Ad)	123
Lecithin/Chol/DSPE-PEG/folate		a.	Inc. in preformed liposomes (Ad)	422

Abbreviations: cationic (c.), hydrophilic (h.), zwitterionic (z.), anionic (a.), incubation (inc.), fusogenic (fuso), adenovirus (Ad), adeno-associated virus (AAV), lentivirus (LV), retrovirus (RV), herpes simplex virus (HSV), murine leukemia viruses (MLV).

for viral gene delivery, which are discussed in the following section are summarized in Table 5.

Formulation. In viral gene delivery, the most frequently used lipids (Chart 2) are cationic phospholipids (*e.g.*, DOTAP, DOSPA and DOTMA), zwitterionic helper lipids (*e.g.*, DOPE) and sterols (*e.g.*, cholesterol (Chol)).[400] The internalization efficiency into cells is highly dependent on the formulation of lipid mixtures.[388,400] Usually the negatively charged viral particles are encapsulated in liposomes and subsequently taken up into cells.[150,400] For example, commercialized transfection agents like Lipofectamine™ and Lipofectin™ contain the mixed formulations DOSPA/DOPE (3/1) and DOTMA/DOPE (1/1), respectively.[400,401] The zwitterionic helper lipid DOPE is added to the formulations to enhance TE by direct fusion with the cell-membrane and bypassing cellular endocytosis, thereby avoiding inefficient endosomal escape.[402] Addition of cholesterol changes at certain concentrations the order of phospholipid assembly and results in more rigid liposomes.[403] However, when pure Chol is applied, it increases infectivity of Ad by forming Chol–Ad clusters *via* hydrophobic interactions with capsid proteins.[404,405]

Formation and release. Complexes of lipids and VP are typically obtained by simple mixing of the VPs in aqueous solution with preformed liposomes on a thin film and subsequent incubation.[406,407] However, concerns regarding this method have been raised due to toxic and transduction ineffective aggregate formation.[408] Alternative encapsulation routes such as spontaneous self-assembly around VPs (Fig. 15A) have been demonstrated to circumvent these problems with partial success.[53,409,410]

In general, it is assumed that TE of VPs is increased by accelerated cell entry due to the small size and positive charge of liposomal carriers[53,410] and further promoted by the so-called proton sponge effect, which has recently been critically

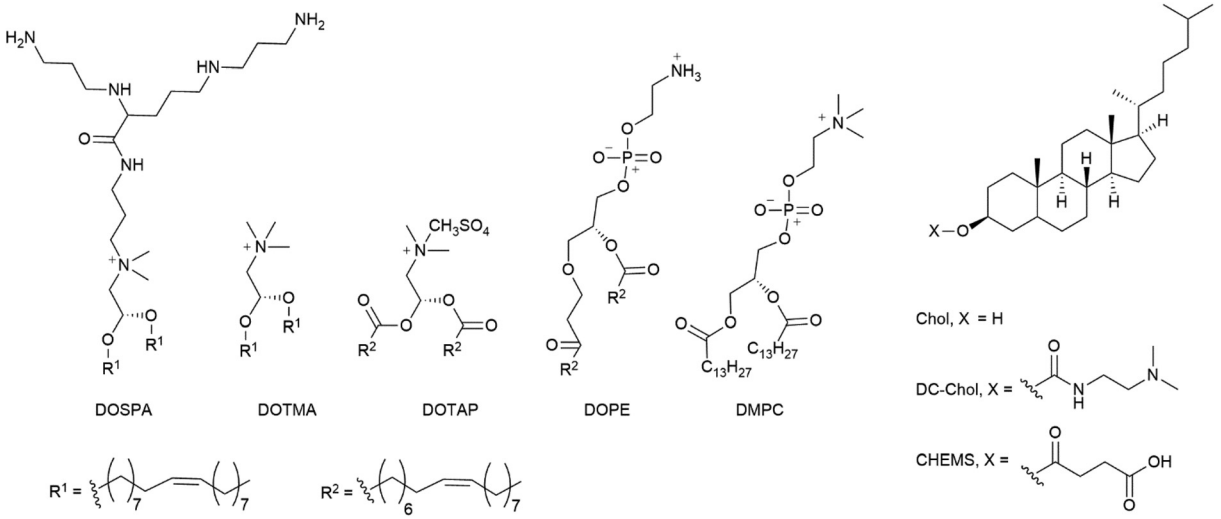

Chart 2 Overview of most frequently used lipids for viral gene delivery.

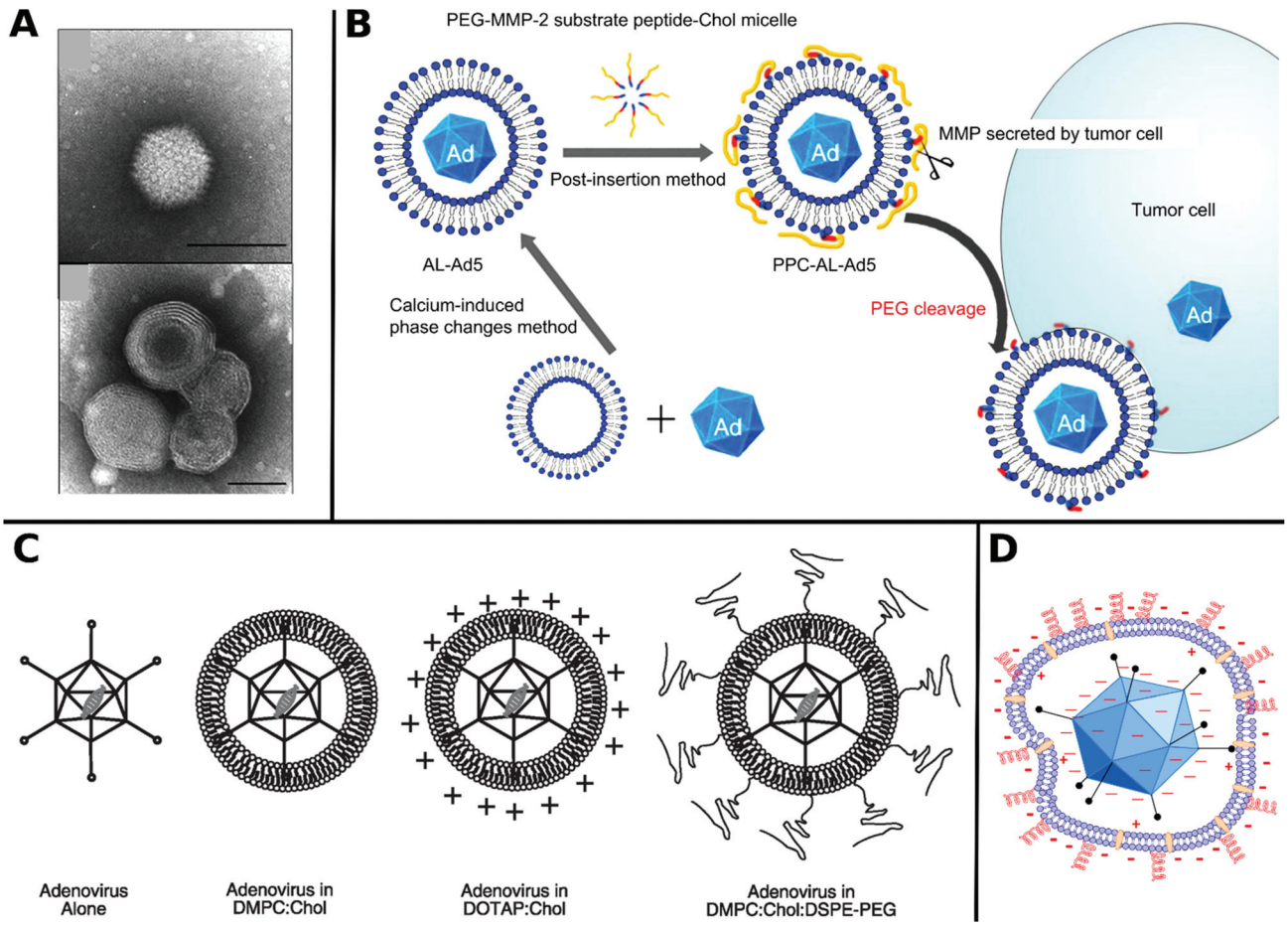

Fig. 15 A. TEM images of naked Ad (top) and DOTAP/Chol encapsulated Ad (bottom) (scale bar: 100 nm). Adapted with permission from ref. 409. Copyright 2008 American Chemical Society. B. Schematic illustration of enzyme-responsive liposome-encapsulated Ad5 for specific delivery in tumor cells. Adapted with permission from ref. 413. Copyright 2013 Elsevier. C. Schematic representation of lipid encapsulated Ad. Adapted with permission from ref. 53. Copyright 2008 John Wiley & Sons. D. Schematic diagram of electrostatic interactions between zwitterionic phospholipid lecithin with negatively charged Ad5. Cholesterol is shown between lipid bilayer and PEG chains on the outer sphere as red spirals. Adapted with permission from ref. 422. Copyright 2014 Elsevier.

reviewed.[195] The proton sponge effect is caused by an increase of cationic charge at endosomal acidic pH, leading to osmotic swelling and accelerated liposome dissociation in the cytoplasm.[129,411] Apart from this, various supporting strategies have been developed to facilitate the endosomal escape by creating stimuli-responsive liposomes, for example, by employing pH-triggered release of Ad from DOPE/CHEMS[412] or MMP-cleavable enzyme-responsive liposomes (Fig. 15B).[413]

Most studies were conducted with complexes from cationic liposomes and VPs. Enhanced TE, shielding from immune response and broadened tropism of RV,[400,401,410,414] AAV,[415] Ad,[124,416,417] HSV,[418,419] as well as recently for oncolytic applications with reovirus[420] and alphavirus[421] were reported. Moreover, liposome vesicles from zwitterionic, cationic and PEG-lipid formulations can act as an artificial envelope for non-enveloped viruses and thereby enhance targeted delivery and prolong blood circulation lifetime (Fig. 15C and D).[53,412,422] Further co-envelopment of Ad and siRNA yielded a dual active hybrid vector for multiple gene delivery.[423]

Not all applications with lipids for virus delivery require preformed VP–lipid complexes. Parsons *et al.* applied lysolecithins as surfactants on host cells prior to LV administration for cystic fibrosis treatment in mice. In this way, a more effective, less immunogenic and persistent transduction was achieved already with a single injection.[424–427] Maguire *et al.* were the first to report and isolate AAV in extracellular vesicles, which were released from AAV producer cells during the reproduction process.[428] These naturally encapsulated AAV showed enhanced TE and high biocompatibility at various application sites *in vivo* and has been reviewed elsewhere.[429]

Modified lipids. One drawback of cationic liposomes in systemic administrations is unwanted interaction with serum proteins and subsequent clearance.[430] To tackle this issue, lipids have been covalently attached to PEG to achieve stealthy carriers with longer circulation times and protection from blood plasma protein adsorption.[166,431] These systems also enable tumor[413,432–434] and cell targeting.[435] In a different approach, PEG spacers were used as surface functionalization tools for liposomes, *e.g.* for covalent attachment of the CPP Tat or Pen.[391,436]

Anionic lipids. Anionic liposomes, on the other hand, were introduced in the last decade and emerged as carriers for VVs with enhanced TE and low toxicity and immunogenicity.[123,437] They are more compatible with the abundance of negatively charged molecules in a physiological environment and therefore less prone to recognition by neutralizing antibodies. Due to the same charge polarity of VPs and anionic liposomes calcium-ions forming bridged complexes were required for encapsulation.[123,437] Surprisingly, enhanced TE is possible despite the anionic charge[438] even in a CAR-independent manner for encapsulated Ad.[123,422,437] The mechanism that promotes interactions of anionic liposomes with plasma membranes is still not fully understood. Possible reasons, such as longer interaction time due to reduced clearance and toxicity,[437,439,440] faster release of cargo,[441,442] or receptor mediated mechanisms[443] have been discussed. A thorough understanding of mechanisms promoting interactions of anionic liposomes with VPs is yet to be achieved.[444,445] This is of significant importance, especially with regard to oncolytic viral gene delivery, where an elevated presence of anionic lipids is observed on cancer cell membrane surface.[446,447]

Nanoparticles

Nanoparticles (NPs) are nanoscale materials, which have attracted growing interest in recent years due to their versatility and unique size-scaling properties. For example, NPs made from noble metals such as gold display strong surface plasmon absorption, making them an interesting material for local phototherapy and as an imaging agent *in vivo*.[448,449] Among manifold preparation techniques to obtain uniform, functional NPs, template-based strategies are well established. VPs are attractive templates due to the uniform, highly precise 3D structure of the virus capsid which are decorated with chemical functional groups.[450]

NPs have also emerged in recent years as an invaluable asset for viral gene delivery. In general three approaches can be distinguished: (1) NPs and VP administered together without specific association between them; (2) VPs and NPs that are covalently bound, *e.g.* in a conjugate; and (3) non-covalent interactions such as electrostatic binding between VPs and NPs. Depending on the NP–VP size ratio, various architectures can be achieved. If the NPs size exceed the VPs size *e.g.* iron oxide NPs combined with AAV[120] VPs are decorated on the outer sphere of NPs. However, if the VPs are larger than NPs typically, NPs and VPs are combined either by coating VP surface *via* incubation and precipitation or by covalent attachment through functional groups on VP surface. For example, mineral shells for VPs can be created by precipitation from a solution of an inorganic salt and exhibit new biological and physical properties impacting stability, host cell adsorption and entry mechanism.[451]

Covalent attachment of NPs to VPs is accessible by abundant lysine residues on capsid proteins and by genetically engineered functional groups, *e.g.* thiol- or azide-moieties.

Frequently applied NPs for viral gene delivery are made from calcium phosphate, gold, silica, graphene or iron oxide, are discussed in the following sections and summarized in Table 6.

Calcium phosphate. Calcium phosphate ($Ca_3(PO_4)_2$) is highly abundant in human bone and teeth and one of the most important biominerals in Nature.[452] As a common material in humans and animals, $Ca_3(PO_4)_2$ has a significant role also for viral infectivity, as reported for avian sarcoma virus. It is suggested that this virus occurs in aves in mineralized state and promotes transmission and bypassing of the zoonotic barrier to humans by a highly effective adsorption and $Ca_3(PO_4)_2$ mediated internalization pathway into host cells.[113] At neutral conditions, $Ca_3(PO_4)_2$ forms a solid precipitate around VPs, which can be degraded under acidic condition present in the endosome.[453] $Ca_3(PO_4)_2$ is an ideal candidate as a biomineral due to its biocompatible properties and degradability into non-toxic ions after cellular entry (Fig. 16). It

Table 6 Overview of nanoparticles utilized for viral delivery, properties and fabrication methods

Name	Structure	Properties	Fabrication of VP–NP vectors	Ref.
$Ca_3(PO_4)_2$	Calcium phosphate	Shell like encapsulation	Coprecipitation by inc. in $CaCl_2$ and subsequent biomineralization through addition of Na_2HPO_4 (Ad5)	112
$Ca_3(PO_4)_2$, DOPA, PEG-DPPE	Calcium phosphate, dioleoylphosphatydic acid, 1,2-dipalmitoyl-sn-*glycero*-3-phosphoethanolamine-*N*-[azido (polyethylene glycol)-2000]	3 layered encapsulation	Coprecipitation in Ca-rich medium, inc. by assembly of lipid bilayers of DOPE, coassembly of PEG-DPPE with DOPE (oAd)	434
$CaCO_3/MnCO_3$		Shell like encapsulation	Coprecipitation from $CaCl_2$ and $MnCl_2$ with Na_2CO_3 (oAd)	454
Gold nanorods		NP = *ca.* 60 × 30 × 1 nm	Coinjection (oAd)	463
Gold NPs	Funct. with PEG/RGD or azide-group	NP = 14 nm	Cov. conj. (Ad)	457
Gold NPs	Au-sulfo-NHS	NP = 1.4 nm	Cov. conj. (Ad)	456
Silica gel	SiO_2 gel polymerized from tetraethoxysilane	Macroporous hydrogel	Cogelation from sol–gel drying process (Ad)	128
Silica cloak	SiO_2	Anionic	Coprecipitation from silic acid in aqueous media on Ad pretreated with PLL	125
SPIONs	Fe_3O_4 (superparamagnetic iron oxide nanoparticles)	NP = 10 nm	Cov. conj. (Ad)/inc. (Ad/measles virus/LV	498, 490, 505, 494, 484 and 493
SPIONs–COOH	Fe_3O_4 functionalized with carboxylic acid	NP = 5 nm	Cov. conj. (AAV)	489, 502 and 503
SPIONs/heparin	Heparin coated SPION	NP = 100 nm	Inc. (AAV)	120
SPIONs/chitosan	Chitosan coated SPION	NP = 50–150 nm	Inc. (Ad)	497
SPIONs/PEG2000	PEG2000 cross-linked SPION	NP = 117 nm	Inc. (Ad)	492
SPIONs/PEI or polybrene or Si-PEI	PEI/polybrene/Si-PEI coatings on SPION	NP = 28 nm/ 64 nm/101 nm	Inc. (oAd)	491
NiNTA-biotin–streptavidin SPIONs	Fe_3O_4 particles conjugated with streptavidin–biotin–nitrilotriacetic acid–Ni	NP = 120 nm	Hexahistidine coding AAV interact with Ni-ions chelated on NPs.	115
PEI–GO–PEG–FA	Polyethyleneimine–graphene oxide sheets–polyethylene glycol–folic acid	NP = 25 nm	Inc. (measles virus)	481
Carbon dots	Carbon dots, citric acid, branched PEI	NP = 11–36 nm	Inc. (rAAV)	482

Abbreviations: incubation (inc.), adenovirus (Ad), oncolytic adenovirus (oAd), adeno-associated virus (AAV), lentivirus (LV).

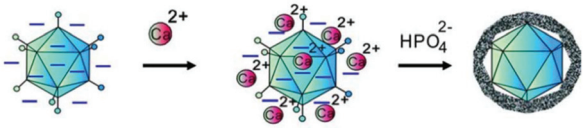

Fig. 16 Schematic illustration of *in situ* mineralization of Ad5 by precipitation with $Ca_3(PO_4)_2$. Adapted with permission from ref. 112. Copyright 2012 John Wiley & Sons.

has been applied as a virus shell by biomineralization and enabled enhanced CAR-independent infectivity, expanded tropism and shielding from neutralizing antibodies.[112,113] Chen *et al.* described a combinatorial design in which oncolytic Ad was subsequently coated with $Ca_3(PO_4)_2$, DOPA lipids and PEG to form a spherical three layered charged shell with almost neutral charge. This design masked the viral surface and lowered unwanted immune response and liver toxicity while improving TE to tumor tissue.[434] Similar, easy to fabricate virus–inorganic complexes from $Ca_3(PO_4)_2$ are promising for systemic administration due to low administration dosage and prolonged circulation lifetime.[112] Recently, Huang *et al.* proposed a more elaborate calcium-based shell for oncolytic Ad delivery, which was created from calcium and manganese carbonates. This shell prolonged *in vivo* circulation of VPs, protected them from immune response, significantly reduced their liver accumulation while still resulting in high tumor accumulation. In the acidic tumor microenvironment, Mn^{2+}-ions were released and converted endogeneous H_2O_2 into O_2. Thereby oncolytic Ad duplication activity and antitumor effects were enhanced. Due to the increase of O_2 concentration, real time, label-free monitoring with magnetic resonance imaging was possible.[454]

Inorganic particles from $Ca_3(PO_4)_2$ can provide stealth biomineral shells by spontaneous coating of VPs surface and thereby have an influence on tropism, TE and biocompatibility. Combinations with other inorganic particles enable precise tuning of the release properties and give access to tailored carriers.

Gold. Gold NPs have been explored for various biomedical applications like diagnostics, radiotherapy or gene delivery due to their easy fabrication, biocompatibility, and functionalization.[455]

Gold NPs were applied as covalent conjugates with VPs and reported either to retain or enhance TE[456,457] or attenuate infectivity,[458–461] depending on the capsid modification. Covalent conjugates were mainly achieved by attachment to the abundant lysine residues of capsid proteins. However,

modifications with high gold amounts interfered with capsid protein functions, which are important for virus infectivity.[456] Nevertheless, enhanced transduction was shown with various positively charged PEGylated Gold NPs functionalized with RGD epitope or azide group and coincubated with Ad, which formed spherical particles and exhibit higher TE to hMSCs compared to PEI or Lipofectamine coated Ad (Fig. 17).[457]

A further interesting clinical application of Gold NPs is photothermal therapy,[462] which has been efficiently addressed in a combinatorial therapeutic approach with VPs.[456] Oncolytic Ad applied in combination with gold nanorods exhibit hyperthermia-induced enhancement of receptor-mediated endocytosis and TE *in vivo*, enabling locally induced tumor cell lysis.[463]

Silica. Silica NPs are biodegradable minerals which are typically fabricated in a sol–gel process starting from tetramethyl orthosilicate or tetraethyl orthosilicate and are broadly employed as food additives or in biomedical applications.[464,465] Silica coatings for VPs are promising candidates to broaden the tropism of viral gene delivery, since a size dependent uptake by tumor cells is known for silica nanoparticles.[466] Recently, Ad coated with silica showed enhanced TE in glioma while reducing liver accumulation and immunogenicity.[125] Other formulations of silica have also been tested. Silica-based gels are synthesized through a simple sol–gel process, resulting in highly porous materials, suitable as implantable viral depots with long-term release. Typically, the degradation of silica gels is in the range of weeks and depends on the amount of water which is present. For example oncolytic Ad were encorporated in a biodegradable high water content silica-based gel and spatiotemporal release of oncolytic Ad in implanted tumor tissue site was achieved by gradually degradation *in vivo.* The virus could be stored in the silica gels and infectivity was preserved for a period of at least two years *in vitro* at 4 °C.[128]

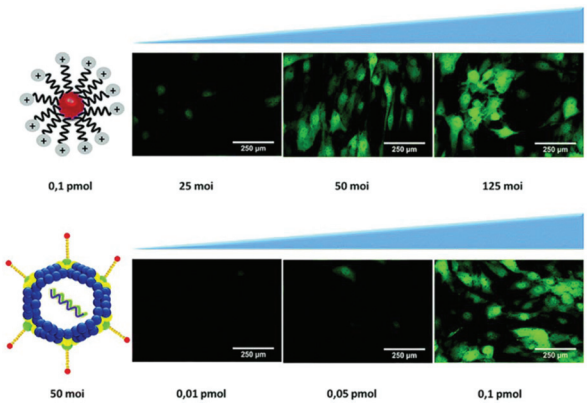

Fig. 17 Optimization process of Ad conjugated to gold NPs by maintaining gold NP concentration fixed at 0.1 pmol and varying amount of VPs (moi, multiplicity of infectivity, top). The best ratio at 50 moi was kept and gold NP concentration was varied (bottom) to observe enhanced TE at higher gold NP concentrations. Adapted with permission from ref. 457. Copyright 2019 The Royal Society of Chemistry.

Graphene. Graphene-based NPs display unique physiochemical properties, like electrical conductivity or photostability and have emerged as an abundant and low-cost resource for nanomedical application in recent years.[467] For example, they have been applied in tumor treatment,[468] photothermal therapeutic approaches,[469–471] virus detection[472] and have been used as drug[473,474] and gene delivery agents.

Since pioneering works from Kostarelos *et al.*[475,476] research efforts have been focussed on graphene as non-viral transfection agents, often in combination with grafted cationic polymers as adhesion promoters.[477–480]

Recently, graphene have started to attract interest as viral delivery platforms. Graphene oxide (GO) sheets decorated with PEG-folate were shown to target susceptible cancer cells.[468] Based on these results, oncolytic measles virus was encapsulated by GO sheets functionalized with PEI and PEG-folate (Fig. 18). The encapsulated measles virus was protected against neutralizing antibodies, showed high tumor accumulation *in vivo*, exhibited higher infectivity and TE to cancer cells than virus alone and folate-free GO–PEI–PEG sheets. However, the release mechanism and *in vivo* stability of the VP–GO sheet cluster remain unexplored.[481]

Another carbon-based material, carbon dots, spherical particles of few nanometer-size diameter, have been applied for viral gene delivery for the first time.[482] This relatively new material class, mostly derived from graphene, has been reported to show size-, charge-, and aggregation-dependent toxicity.[483] In order to stimulate cartilage repair, rAAV coding for the highly chondroreparative SOX9 transcription factor was complexed with various functionalized carbon dots, which resulted in significant increase of transduction to hMSCs with high cell viability. Especially carbon dots functionalized with 2-citric acid, poly(ethylene glycol) monomethyl ether and *N*,*N*-dimethylethylenediamine displayed efficient release and TE of rAAV.[482]

Iron oxide. Iron oxide NPs have an outstanding position among inorganic NPs for viral gene delivery and have been intensively studied as an interface technique between physical and chemical delivery. They have especially attracted interest as the main component for remotely guided magnetic delivery vehicles. Gene delivery by using a gradient magnetic field, also

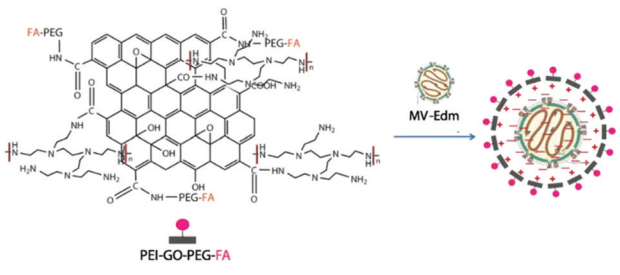

Fig. 18 Structure of PEI–GO–PEG–FA and schematic illustration of GOS/measles virus (MV-Edm) complexation for cancer therapy. Adapted with permission from ref. 481. Creative Commons BY 4.0. 2019 Springer Nature.

called magnetofection, has been reported to enhance TE of non-viral vectors and VVs after employing external magnetic fields for *in vitro* and *in vivo* applications[484–486] and have been successfully commercialized for example as AdenoMag (OZ Biosciences),[487] fluidMAG-Heparin (Chemicell)[120] or Endorem (Guerbet).[488]

Magnetite (Fe_3O_4) as superparamagnetic iron oxide nanoparticles (SPIONs) have been reported in several studies as a vehicle to enhance gene delivery efficiencies, *e.g.* for delivery of AAV,[115,489] Ad,[487,490] oncolytic Ad,[491,492] LV,[484,493] and measles virus.[494] They are biocompatible and degradable and allow surface modification with various functionalities.[495,496]

Coating SPIONs with biopolymers like heparin[120] or chitosan[497] can further augment TE with lower vector dosage and enable faster transduction (Fig. 19A). Moreover, polymeric coatings such as PEI or PEG on SPIONs as well as liposome encapsulation are reported to be highly beneficial for improving TE of Ad with reduced dose (Fig. 19B).[484,492,498] For example, PEI coatings give access to positively charged particles boosting cell interaction and endosomal escape by their proton sponge effect without affecting the superparamagnetic properties.[499] In a comparative study PEI, polybrene, or silica–PEI were coated onto iron oxide NPs and resulted in improved potency of oncolytic Ad. The highest antitumor activity *in vivo* was observed for silica–PEI coated iron oxide NPs, which was attributed to a higher shielding ability against neutralizing antibodies and higher magnetophoretic mobility.[491] In another study, various commercially available SPIONs were compared to each other in terms of susceptibility for aggregation and TE of attached alphavirus. Positively-charged NPs displayed rapid and effective isolation and concentration of VPs, resulting in higher TE than negatively charged NPs.[500]

In general, VPs can be coated with modified SPIONs through simple incubation due to electrostatic interactions to obtain hybrid particles.[487,492] Beside this, covalent attachment of VPs onto SPIONs is regularly achieved by EDC–NHS chemistry coupling carboxy-functionalized SPIONs to the lysine residues on viral capsid.[489,498]

Another, more laborious route to attach VPs onto SPIONs surfaces can be achieved by using a chelating approach, with a linker unit consisting of biotin-functionalized nickel ions conjugated to streptavidin modified SPIONs, which further bind to hexahistidine displaying AAV particles (Fig. 19C). This hybrid particle can transduce into otherwise non-permissive hNSCs by employing magnetic force.[115] Recently, precise manual virus stamping to single cells in culture as well as to mouse brain was reported by Schubert *et al.* This technique employs VPs covalently bound to silica-coated SPIONs which are loaded in a micropipette tip and brought into contact with the desired cells by a magnetic field (Fig. 19D). This method enables the delivery of multiple virus types to a single cell for multiple virus engineering or to track genetically modified single cells.[501]

An interesting example to implement magnetofection with phototherapeutic tumor treatment was shown for AAV encoding a photosensitive apoptosis-inducing protein. AAV conju-

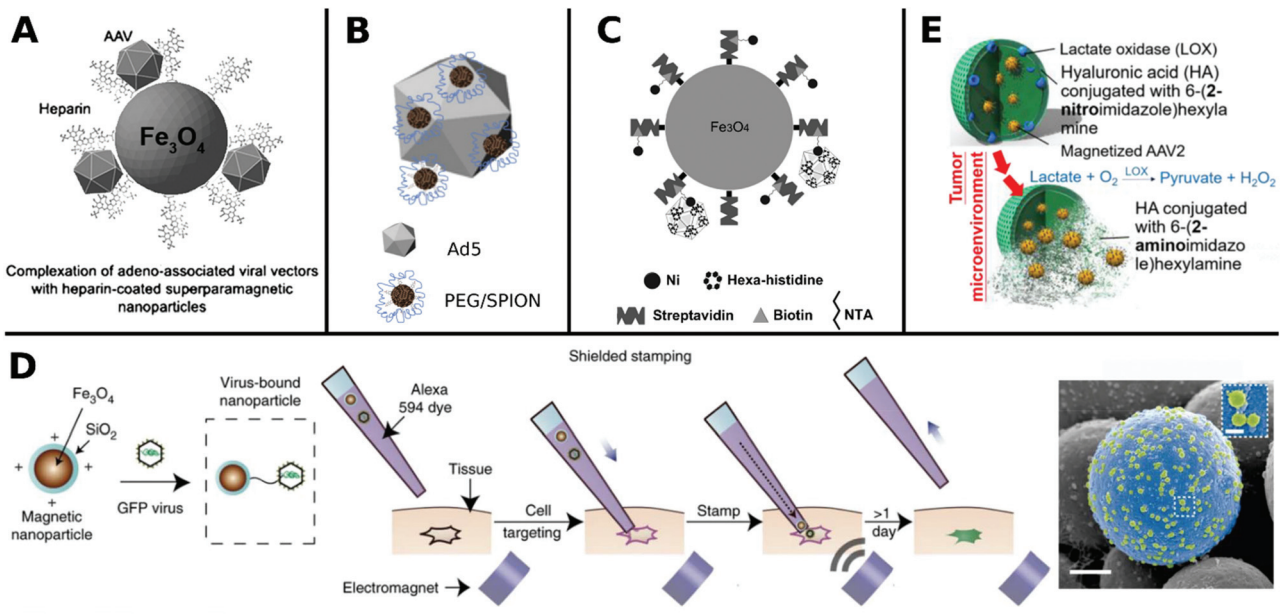

Fig. 19 A. Schematic illustration of AAV bound to heparin-coated SPIONs. Adapted with permission from ref. 120. Copyright 2011 Elsevier. B. Schematic illustrations of PEG_8-functionalized SPION NPs-labeled Ad5 capsids. Adapted with permission from ref. 498. Copyright 2012 Elsevier. C. Schematic illustration of streptavidin functionalized SPIONs chelated with biotin bound hexahistidine/AAV. Adapted with permission from ref. 115. Copyright 2011 Elsevier. D. Schematic representations of the main steps for single-cell virus stamping and colored SEM image of magnetic NP (blue) bound to LV (green) (scale bar: 500 nm, inset: 100 nm). Adapted with permission from ref. 501. Copyright 2018 Springer Nature. E. Tumor bio-reduction-activated NPs incorporating magnetized virus delivery. In the reductive tumor microenvironment the carrier payload dissociates and AAV conjugated SPIONs can be tracked by MRI. Adapted with permission from ref. 503. Copyright 2018 American Chemical Society.

gated to iron oxide NPs were remotely guided and delivered to the tumor site with microscale precision, where phototoxicity was then triggered by irradiation.[489,502,503] Recently, Tseng *et al.* further developed this approach with an elaborate hybrid design. AAV encoding the photosensitive apoptosis-inducing protein was conjugated to SPIONs and loaded to a nitroimidazole conjugated hyaluronic acid NP containing lactate oxidase. This design employed the lactate-rich tumor microenviroment to subsequently initiate H_2O_2 production by lactate oxidase, resulting in reduction and disassembly of 2-nitroamidazole-hyaluronic acid matrix and finally release of SPIONs attached to AAV into tumor site (Fig. 19E). This enzymatically-controlled release enabled targeted tumor cell lysis with an combinatorial approach of functional materials.[503]

SPIONs are approved as contrast agents for magnetic resonance imaging,[504] which makes them a clinically promising vehicle for targeted delivery of VPs. One of main advantages of using magnetic NPs for VP delivery is the reduced vector dosage and short exposure times for efficient transduction due to controlled colocalization of cells and VPs. Magnetic NPs enable to deliver VPs to otherwise non-permissible cells and may enable the crossing of biophysical barriers *in vivo* in the future.

Small molecules

Small molecules are defined as organic compounds with a molecular mass below 900 Da and a physical size below 1 nm in pharmaceutical research. Today, the majority of drugs used as medicine in everyday life are small molecules, which are characterized by their high bioavailability and effective mode of action at the target site.[81] Research on small molecules that promote viral gene delivery has become more wide-spread in recent years.

The aim of adding small molecules during a transduction procedure is to achieve satisfactory TE with low vector doses to minimize side-effects and to enable transduction to cell types that are difficult to infect. The underlying mode of action of small molecules to achieve this aim is fundamentally different to previously discussed materials, which were interacting with VPs, forming a complex, aggregate or conjugate which in turn facilitates the interaction with cells. Here, small molecules interfere with multiple cellular processes, for example, by affecting gene transcription, cell entry mechanisms or DNA replication and thereby facilitating viral gene expression. The focus in small molecule research for viral gene delivery has been on dual mode drugs, which are promising due to their therapeutic as well as transduction enhancing properties. These molecules are usually simply added to VPs for application, even though covalent conjugation to VPs were also shown exemplarily.[506] In the following sections important findings are highlighted and summarized in Table 7 and Chart 3.

In early works, Miller *et al.* showed that DNA-damaging agents like UV-irradiation or cisplatin increased TE of AAV to non-dividing cells.[507] Later they introduced less cytotoxic DNA-synthesis inhibitors like aphidicolin or hydroxyurea and topoisomerase inhibitors such as etoposide or camptothecin as enhancers of AAV transduction to fibroblasts.[508] Groschel *et al.* demonstrated that these cytostatic agents etoposide, camptothecin, taxol, and aphidicolin, interfere with various mechanisms of cell cycle progression and arrest the cell cycle at G_2/M phase, which is a checkpoint to control DNA replication before nuclei division. Arresting the cell cycle in this phase boosted the early step of HIV virus replication and thereby enhanced TE.[135] Another well-known chemotherapeutic drug, doxorubicin, greatly enhanced rAAV infection to airway cells as well as neuronal cells with only mild cytotoxic effects at tested concentrations. Doxorubicin promoted rAAV accumulation at the nucleus, probably through proteasome inhibiting effects resulting in accelerated intracellular viral nuclear transport, but without affecting cell entry mechanisms.[509,510] The exact mechanism remained unclear and it was suggested that enhanced TE is more likely resulting from stimulated capsid uncoating and increased reverse transcription of viral genome than by increased cell entry.[135] Further cytokine stimulated downregulation of proteasome activity is believed to enhance TE.[511] Evidence for this hypothesis was found by addition of cytokines or proteasome inhibitors, particularly FDA approved bortezomib, resulting in enhanced TE of even low dosed LV to stem cells.[511–513]

Furthermore, the proteasome inhibitor MG132 (carbobenzoxy-L-leucyl-L-leucyl-L-leucinal) enhanced TE of rAAV to airway epithelia cells by modulating the ubiquitin–proteasome system in the endosomal environment.[514] Moreover, adenosine 5′-triphosphate-binding cassette transporter proteins, which efflux chemotherapeutic drugs and HIV protease inhibitors, play a significant role in reducing TE of LV into $CD34^+$ stem cells. Enhanced TE of up to sixfold was observed after the addition of Verapamil, an adenosine 5′-triphosphate-binding cassette-inhibitor.[515]

However, the identified chemotherapeutic compounds have huge disadvantages including damaging DNA and are therefore not ideal candidates for clinical use to healthy cells. In order to identify additional transduction enhancing drugs, high-throughput screens were conducted.

The first library screening for compounds promoting LV transduction identified 30 potential enhancers of viral transduction from 1280 investigated pharmaceutically active drugs. The non-DNA damaging, protein kinase activator phorbol 12-myristate 13-acetate (PMA) was found as a very promising candidate to enhance TE of LV into hard to transduce stem cells. Similar to previously discovered agents, PMA displayed interference with the cell cycle and inhibition of proliferation, suggesting that the transduction efficiency is influenced by processes within the cell rather than by promoted cell entry.[516]

Another high-throughput screening with focus on enhancing TE of rAAV to HeLa cells investigated 2396 pharmaceutically known, mostly FDA approved compounds from which 13 compounds were identified as capable potentiators. In this screening, five main enhancer groups were identified: epipodophyllotoxins, inducers of DNA damage, effectors of epigenetic modification, anthracyclines, and proteasome inhibitors.

Table 7 Overview of drugs presented for viral delivery, properties and fabrication methods

Name	Medical use	Assumed mechanism of action	VPs and cell line	Ref.
Camptothecin	Cytostatic drug	Topoisomerase inhibitor/G_2/M cell cycle arrest	AAV to fibroblasts/HIV to HeLa	508 and 135
Taxol	Cytostatic drug	G_2/M cell cycle arrest	HIV to HeLa/AAV to HeLa	135 and 506
Hydroxyurea	Cytostatic drug	DNA-synthesis inhibitor	AAV to fibroblasts	508
Aphidicolin	Cytostatic drug	DNA-synthesis inhibitor	AAV to fibroblasts/HIV to HeLa	135 and 508
Verapamil	Calcium channel blocker	Adenosine 5′-triphosphate-binding cassette-inhibitor	Vesicular stomatitis virus-G to hematopoietic progenitor cells	515
Bortezomib	Cytostatic drug	Proteasome inhibitor	Lymphocytic choriomeningitis virus (LCMV) to T-cells/rAAV to HeLa, U87, HepG2, NHF1	513 and 517
MG132		Proteasome inhibitor	rAAV to human airway epithelia	514
Phorbol 12-myristate 13-acetate	Proteinkinase activator		LV to HSC	516
Doxorubicin	Cytostatic drug	Proteasome inhibitior	rAAV to neuronal cells/rAAV to human airway epithelia	510, 509 and 527
Teniposide	Cytostatic drug	Topoisomerase inhibitior	rAAV to HeLa, U87, HepG2, NHF1	517
Etoposide	Cytostatic drug	Topoisomerase inhibitor	rAAV to HeLa/AAV to fibroblasts/HIV to HeLa	517, 508 and 135
Bleomycin	Cytostatic drug, glycopeptide antibiotic	DNA damage	rAAV to HeLa, U87, HepG2, NHF1	517
Parthenin	Sesquiterpene lactone		rAAV to HeLa, U87, HepG2, NHF1	517
RH-1	Diaziridinylbenzoquinone		rAAV to HeLa, U87, HepG2, NHF1	517
Vorinostat	Cytostatic drug	HDAC inhibition	rAAV to HeLa, U87, HepG2, NHF1	517
Nanaomycin A	DNMT3B inhibitor	DNMT3B inhibitor	rAAV to HeLa, U87, HepG2, NHF1	517
Menogaril	Anthracycline		rAAV to HeLa, U87, HepG2, NHF1	517
Pyrromycin			rAAV to HeLa, U87, HepG2, NHF1	517
Daunorubicin		DNA intercalation, topoisomerase inhibition, polymerase inhibition, free radical damage to DNA	rAAV to HeLa, U87, HepG2, NHF1	517
Physalin B			rAAV to HeLa, U87, HepG2, NHF1	517
Siomycin	Thiazole antibiotic		rAAV to HeLa, U87, HepG2, NHF1	517
Tetrocarcin A	Microbial metabolite	BCL-2 inhibitor	rAAV to HeLa, U87, HepG2, NHF1	517
6-Gingerol	Phytoactive material, anticancer activity	Bypassing endocytic receptor mediated pathway, interfering with cell cycle	AAV2- to A375 and 526 malignant melanoma cells	58
Pyrrol based derivatives		Inhibition of production of IFNβ and various interferon-stimulated genes	Oncolytic Rhabodviruses to resistant cancer cells (*e.g.* CT26)	518
Prostaglandine E_2	Abortifacient agent, labor induction		LV to HSCs	520–522
(+)-JQ1	Cytostatic drug	Bromodomain inhibitor	Ad to A549, HeLa, Jurkat and P388D1 cells	524
Hydroxychloroquine	Antimalaria medication	TLR9 inhibition	AAV to murine and human tissue	523
Rosuvastatin	Statin medication	Promoter of low-density lipoprotein-receptor	Vesicular stomatitis virus (LV) to natural killer cells	526
Embelin	Cytostatic drug and anti-inflammatory effects	Inhibitor of X chromosome-linked inhibitor-of-apoptosis protein XIAP	Oncolytic vaccinia virus to T cells and NK cells	525

The highest level of transduction, with approx. 50–100 times compared with virus-only transduction, was observed for the chemotherapeutic epipodophyllotoxin teniposide. It was observed that the TE varied significantly for the tested substances depending on cell type, which indicates an underlying correlation between mode-of-action and cell type. In unison with previous studies, the authors suggest that rAAV can be enhanced by two pathways, whose detailed mechanisms remain unknown: the inhibition of proteasomes and the induction of DNA damage.[517]

A further screening for enhancing interferon sensitized oncolytic virus transduction to resistant cancer cells identified pyrrole-based molecules as potent enhancers. This was also feasible with AAV and Ad. High activities up to thousand-fold enhancement of viral transduction, tolerability and plasma stability were found for systemic administration in mice. The mode of action of these molecules was attributed to an inhibition of interferon-β production, which gives antiviral properties to resistant cancer cells.[518,519]

In recent years, further pharmaceutically applied drugs were identified as promising viral gene delivery agents.

Prostaglandine E_2 enhanced LV transduction to HSCs by acting during the endocytosis phase *via* a yet unclear mechanism.[520–522]

Lee *et al.* reported synergistic effects in terms of inducing apoptosis to malignant melanoma cells when AAV2 encoding a pro-apoptotic protein and the phytoactive compound 6-gingerol were combined. 6-Gingerol was previously reported to be

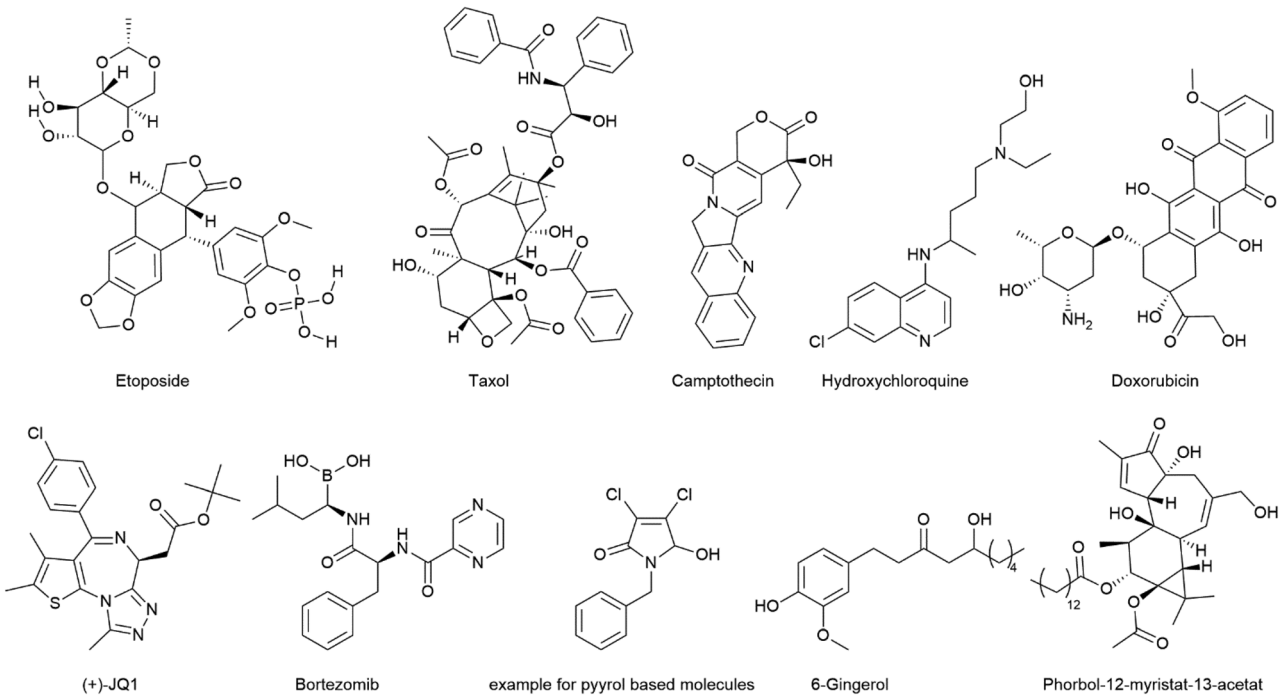

Chart 3 Overview of selected drugs as enhancers of viral gene transfer.

effective against cancer and for the inhibition of angiogenesis. The cytostatic activity has its source in the control of the cell cycle and subsequently inducing apoptosis. The authors proposed that AAV2 can bypass its usual endocytic receptor-mediated pathway in the presence of 6-gingerol and thereby enhancing apoptosis in melanoma cells more efficiently.[58]

Furthermore, hydroxychloroquine, an approved antimalaria drug, has been applied for promoting AAV transduction in murine and human tissue *in vitro* and *in vivo*, *e.g.* in the retina with no adverse effects. Among the multimodal effects this drug might have on TE, one mode of action was proposed as an inhibition of TLR9 gene expression, which is a known sensor for anti-viral response of cells.[523]

Intervention in cell regulation processes was similarly shown for Ad infection which was enantioselectively promoted by the bromodomain inhibitor (+)-JQ1. This molecule blocks acetylated lysine interaction with the bromodomain family proteins, which play a significant role in cell regulation and gene transcription. In this way, Ad infectivity and gene expression were elevated by suppression of regulating bromodomains, whose mechanisms still need to be elucidated.[524]

Embelin, used as an adjuvant in oncolytic virotherapy was recently reported by Wang *et al.* Lymphoma cell lysis was induced by mitigating antiviral immunity against oncolytic virus upon addition of Embelin. The authors proposed that the infiltration of the oncolytic virus was facilitated by the disruption of Interleukin-6/STAT3 signalling and further promoted by the inherent ability of Embelin to induce apoptotic death to tumor cells.[525]

An interesting approach was reported by Gong *et al.* who enhanced TE by increasing low-density lipoprotein-receptors expression with Rosuvastatin. Vesicular stomatitis virus was able to efficiently target and transduce in natural killer cells after addition of Rosuvastatin. Naturally, untreated killer cells lack of sufficient low-density lipoprotein-receptors, which are necessary for virus interaction. Statins, especially Rosuvastatin, can induce low-density lipoprotein expression and thereby facilitate virus–cell interaction. Additionally, geranylgeranyl-pyrophosphate suppresses cytotoxic effects of statins, resulting in a highly effective and powerful tool to enable genetic modification of natural killer cells. Genetically modified natural killer cells can, for example, express tumor antigen-specific receptors, which may pave the way for targeted treatment of malignant cells.[526]

Small molecules, which are beneficial for disease treatment, eliciting host immune response while enabling efficient transduction are actively being pursued for clinical application. Pharmaceutically active drugs that have been found so far, are paving the way especially for oncolytic gene therapy and can be included easily in *ex vivo* gene transfer protocols. However, further research is indispensable for investigation of unwanted side effects of drugs combined with viruses during delivery and should be carefully evaluated in therapeutic application.

From development to usage

Most of the introduced materials for promoting viral gene transfer have not yet been translated to the clinic and are often not commercially available. Here, we will highlight materials that are emerging from the development stage to actual clini-

cal usage. Various material classes which have the potential for application in medical formulations are currently investigated in preclinical studies. For example, *in vivo* studies in animals utilized materials like PLGA,[133,155,215,216] PEG,[163,181,283] PEI,[208] poloxamers,[160,229,231,232,238,239] dendrimers,[116,251,253] polysaccharides,[258,259,262,265] gelatin,[385,386] collagen,[126] fibrin,[374] human serum albumin,[375,377] calcium carbonate,[454] gold nanorods,[454] silica implants,[128] graphene,[481] magnetic NPs,[491,501,505] and small molecules.[517,518,523]

Commercially available kits make transduction enhancers more broadly available and are thus attractive tools for use in research. For instance, several studies about CAR T-cell manufacturing employed kits of the peptidic enhancers Vectofusin®-1 (Miltenyi Biotec) and Protransduzin™ (JPT Peptide Technologies).[353,354,528,529] However, popular materials in research cannot seamlessly be transferred to the clinical context. Low cell viability in high concentrations limits the application of Polybrene® (Abbott Laboratories Corp.) in drug formulations.[530] More efficient enhancers than polybrene[531] are traded as jetPEI® (Polyplus transfection) and Lipofectamine™ (Thermo Fisher Scientific) and can be applied to a broad range of VVs. Kits for specific viruses are available, but their exact composition is a trade secret, *e.g.*: for LVs (ViraDuctin™ (Cell Biolabs), TransDux™ (System Biosciences), ViralPlus (Applied Biological Materials Inc.), SureENTRY (Qiagen)), as well as for Ad in the form of AdenoBoost™ (Sirion Biotech). High transduction rates with low-titer viruses are enabled with polycationic magnetic beads, commercialized as MACSductin™ (Miltenyi Biotec), ViroMag and AdenoMag (OZ Biosciences), Lenti-X™ Accelerator (Takara Bio) and MISSION® ExpressMag® (Sigma-Aldrich). In recent years, a growing number of such kits were made available in GMP grade addressing the demand for systematic investigation of transduction agents in a clinical context.

Clinical trials use RetroNectin® (Takara Bio) in particular as the gold standard for *ex vivo* transduction of lenti- and retroviruses, even though a high multiplicity of infection and a time consuming coating protocol is needed.[361,532–534] Poloxamers such as LentiBoost™ (Sirion Biotech)[535] are commonly used in formulations for *in vivo* clinical trials as enhancers and to prevent uncontrolled binding of VVs to injection devices.[536–539] While the majority of viral gene therapy drugs approved up until 2017 do not use additional transduction enhancers in their formulations, those approved since then have made use of agents such as protamine sulfate (*ex vivo*, Zynteglo),[540,541] Poloxamer 188 (*in vivo*, Zolgensma and Luxturna),[542,543] and human serum albumin (*ex vivo*, Yescarta, Kymriah).[544,545]

Conclusions and outlook

Viral gene delivery has come a long way and is now becoming increasingly therapeutically relevant. The rising number of approvals for clinical use in recent years holds a great promise for treatment of currently uncurable diseases. However, the full potential of gene therapy cannot be realized until safe and efficient delivery systems are available. In this review, we highlighted the most important approaches for viral gene delivery from a materials perspective. In particular, the high biocompatibility of peptidic delivery systems, and the superior shielding effects of polymer coatings and lipid encapsulation need to be emphasized. Both hydrogels and magnetic NPs allow spatially defined virus release, often in a time-controlled manner. Especially the local targeted delivery of VVs *via* magnetic nanoparticles to tumor sites has enormous potential in oncolytic virotherapy. The performance of delivery systems is determined by many experimental parameters, *e.g.* virus type, cell type, concentration, and incubation time. However, detailed large-scale comparative studies of different delivery agents are still missing. Therefore, it is often difficult or even impossible to draw conclusions on the relative performance of the delivery agents. In current viral gene delivery systems, immunogenicity, native tropism, unspecific delivery, and poor efficiency at low vector doses are remaining hurdles to be overcome. The materials introduced here have the potential to establish hybrid VVs for clinical applications. However, clinical data of administration to humans remains scarce.

Gene delivery as a therapeutic approach is one of the major medicinal and biotechnological advances of this century. We expect that novel bioengineered viruses promoted by synthetic materials are destined for future efficient and safe clinical applications.

Conflicts of interest

There are no conflicts to declare.

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