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Cyclodextrins (CDs) are naturally occurring cyclic oligosaccharides. They consist of (α,1,4)-linked glucose units, and possess a basket-shaped topology with an “inner—outer” amphiphilic character. Over the years, substantial efforts have been undertaken to investigate the possible use of CDs in drug delivery and controlled drug release, yet the potential of CDs in gene delivery has received comparatively less discussion in the literature. In this article, we will first discuss the properties of CDs for gene delivery, followed by a synopsis of the use of CDs in development and modification of non-viral gene carriers. Finally, areas that are noteworthy in CD-based gene delivery will be highlighted for future research. Due to the application prospects of CDs, it is anticipated that CDs will continue to emerge as an important tool for vector development, and will play significant roles in facilitating non-viral gene delivery in the forthcoming decades.
of the mRNA coding for the spike protein, and contains the intergenic consensus sequence of an entropic coronavirus) against viral growth in human adenocarcinoma cells [22]. They discovered that compared to the naked OD which resulted in only 12–34% of viral inhibition in vitro, up to 90% of viral inhibition could be obtained when the OD was complexed with an β-CD derivative, 6-deoxy-6-β-D-galactopyranosyl-6-thio-cyclomalto-heptaose, in a molar ratio of 1:100 [23–27]. This, along with other studies [27,28], has paved the way for subsequent intense research on CD-mediated gene delivery.

![Image of CD structures](image.png)

**Fig. 1.** The structures and space-filling models of (i) α-CD, (ii) β-CD and (iii) γ-CD. In the space-filling models, hydrogen, carbon and oxygen atoms are colored in black, white and gray, respectively.

|                           | α-CD                                | β-CD                                | γ-CD                                |
|---------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| **Other names**           | Cyclohexaamylose; α-Schardinger dextrin; Cyclomaltohexaose | Cycloheptaamylose; β-Schardinger dextrin; Cyclomaltoheptaose | Cyclooctaamylose; γ-Schardinger dextrin; Cyclomaltooctose |
| **Physical appearance**   | White powder                        | White powder                        | White powder                        |
| **Odor**                  | Odorless                            | Odorless                            | Odorless                            |
| **Number of α-D-glucopyranose units** | 6                                   | 7                                   | 8                                   |
| **Empirical formula**     | C₆₀H₆₀O₃₀                           | C₆₂H₇₀O₃₅                           | C₆₄H₈₀O₄₀                           |
| **Molecular weight (Da)** | 972                                 | 1135                                | 1297                                |
| **Outer diameter (Å)**    | 14.6                                | 15.4                                | 17.5                                |
| **Cavity diameter (Å)**   | 4.7–5.3                             | 6.0–6.5                             | 7.5–8.3                             |
| **Height of torus (Å)**   | 7.9                                 | 7.9                                 | 7.9                                 |
| **Cavity volume (Å³)**    | 174                                 | 262                                 | 427                                 |
| **Solubility in water (g/L)** | 1.5                                 | 0.2                                 | 2.4                                 |

The information listed in this table is partly based on the following references: [1,14,24].
CDs can be appealing to gene delivery applications because not only of their binding affinity to nucleic acids [17,29] but also of their ability to attenuate the cytotoxicity of other gene carriers. The latter has been supported by a previous study [30], in which a series of polycationic amphiphilic CDs (paCDs) were constructed via condensation of diamino-CD monomers with diimidate co-linear cationic has been supported by a previous study [30], in which a series of polycationic amphiphilic CDs (paCDs) were constructed via condensation of diamino-CD monomers with diimidate co-linear cationic groups. Compared with polyamidines lacking CDs, the IC50 of the βCDs to BHK-21 cells were remarkably higher [30]. This showed that CD incorporation into the backbone of the cationic polymer substantially lowers the polymer cytotoxicity. In addition to the properties mentioned above, CDs are effective absorption enhancers in therapeutics delivery. As illustrated in vitro by skin permeation studies [31], after complexation with β-CD, meglumine antimoniate (MA) led to a 2-fold increase in the antimony flux. A similar absorption-enhancing effect of CDs was shown by using dimethyl-β-CD, which, at a concentration of 5% (w/v), elevated the permeability of the nasal mucosa to the intranasally administered neurotrophic peptide, Org2766, and enhanced the absorption in rabbits 1- to 2-fold from 10 ± 6% (mean ± s.d.) for administration of the peptide alone to 17 ± 8%, and in rats 5-fold from 13 ± 4% to 65 ± 21% [32]. All these evidenced the absorption-enhancing property of CDs, and it is this property that may also facilitate gene delivery. The latter has been substantiated by an earlier study, which successfully improved adenoviral-mediated gene transfer to the rat jejunum by using CDs [33]. The improvement has been ascribed to the CD-mediated enhancement of viral binding and internalization into the host cells.

In fact, CDs have comparatively large molecular weights (>972 Da) and low octanol/water partition coefficients. This, along with the presence of a plurality of hydrogen donors and acceptors on their molecules, has made CDs unlikely to be directly permeable to lipophilic biological membranes such as skin and gastrointestinal mucosa [34–37]. It was hypothesized that CDs enhance absorption mainly by increasing membrane permeability through complexation with membrane phospholipids and cholesterol [38]. This hypothesis was supported by an earlier study, which depleted membrane cholesterol from porcine, bovine and human erythrocytes by incubating the cells in suspensions of lecithin liposomes [39]. The study found that membrane permeability remained unaltered when the level of cholesterol removal was up to 30%, but upon more extensive cholesterol depletion, the transfer rates of nonelectrolytes and organic acids penetrating the membrane were considerably elevated [39]. However, the biphasic response of cholesterol depletion on membrane permeability in erythrocytes appeared not to be reproducible in artificial lipid membranes [39]. The effect of cholesterol depletion on membrane permeability is still obscure, and further study is required before the molecular basis of CD-mediated absorption enhancement can be fully elucidated.

### 3. Applications in gene delivery

CDs have practical potential in gene delivery, but due to their failure to form stable complexes with plasmid DNA (pDNA) [40], native CDs have limited transfection efficiency. CDs are, therefore, usually derivatized prior to their use in gene transfer. A good example of CD derivatives is polycationic amphiphilic CDs (paCDs) (which were constructed by the amendment of the facial anisotropy of the truncated-cone CD torus via instillation of cationic and

### Table 2

| Drug name                  | General use                                                                 | CD for complexation | Trade name(s) of the formulation(s) |
|----------------------------|------------------------------------------------------------------------------|---------------------|-------------------------------------|
| Cefotiam hexetil hydrochloride | An antibiotic that works against pathologic organisms and parasites | β-CD                | Pansporin T                         |
| Limaprost                  | A prostaglandin E1 receptor agonist used to increase blood flow and to inhibit platelet aggregation | β-CD                | Opalmon, Prorenal                   |
| Prostaglandin E1           | A prostaglandin E1 receptor agonist used to maintain a patent ductus arteriosus in newborns, to treat erectile dysfunction, and to tackle critical limb ischemia | β-CD                | Edex, Caverject, Prostavastin, Rigidur |
| Benexate                   | A drug used to treat gastric ulcer                                           | β-CD                | Lommel, Ulgut                       |
| Dexamethasone              | A glucocorticoid receptor agonist with anti-inflammatory and immunosuppressant properties | β-CD                | Glymesason                          |
| Dinoprostone               | A prostaglandin E2 receptor agonist used as a vaginal suppository to prepare the cervix for labour and to induce labour | β-CD                | Prostarmon E                        |
| Iodine                     | A topically applied anti-infective agent                                     | β-CD                | Mena-Gargle                         |
| Nicotine                   | A smoking cessation adjunct                                                  | β-CD                | Nicorette, Nicogum                  |
| Nimesulide                 | A cyclooxygenase-2 inhibitor with anti-inflammatory and anti-rheumatic properties | β-CD                | Nimedex, Mesulid                    |
| Nitroglycerin              | A vasodilator used to treat heart conditions (such as chronic heart failure and angina pectoris) | β-CD                | Nitopen                             |
| Omeprazole                 | A proton pump inhibitor used to treat peptic ulcer and other diseases (e.g. dyspepsia, gastroesophageal reflux, laryngopharyngeal reflex, and Zollinger–Ellison syndrome) | β-CD                | Omebeta                             |
| Piroxicam                  | A non-steroidal anti-inflammatory drug with analgesic and antiinflammatory properties | β-CD                | Brexin, Flogene                     |
| Tiaprofenic acid           | A non-steroidal anti-inflammatory drug with analgesic properties             | β-CD                | Surgamyl                            |

The information listed in this table is partly based on the following references: [13,15,16,24,35].
hydrophobic elements in the “skirt” or “jellyfish” architectures [41,42]. By fine adjustment of the molecular parameters (e.g. charge density, hydrophilic-hydrophobic balance, nature of the functional groups, and spacer length), the DNA complexation capacity and transfection efficiency of paCDs can be modulated [43–45]. More examples of CD derivatives are shown in Fig. 2. They were fabricated by modification of β-CD with a pyridylamino, alkylimidazole, methoxyethylamino or primary amine group at the 6-position of the glucose units [40]. Studies with 32P-labeled pDNA indicated that these derivatives promoted cellular uptake of the transgene much more efficiently than native CDs [40]. Among these derivatives, those having unmodified 2- and 3-hydroxyls and possessing an amino, pyridylamino or butylimidazole group at the 6-position were found to have the best performance in COS-7 cell transfection [40]. These molecular constructs warrant further development as gene carriers. Aside from being used directly for gene delivery after derivatization, CDs have been used as linking agents or structural modifiers for development of gene carriers.

3.1. Functioning as linking agents

By functioning as linking agents, CDs are used to covalently link other polymers together to form larger molecular constructs as gene carriers. One example of polymers fabricated by this approach was synthesized by linking low molecular weight poly(ethyleneimine) (PEI), which is a cationic aziridine polymer exhibiting a high proton buffering capacity over a broad range of pH [46], with β-CD by using tosyl chloride to first generate amine-reactive tosyldeoxy-β-CD, which subsequently reacted with PEI to generate the CD-PEI conjugate (PEI-β-CD) [25]. In vitro studies showed that PEI-β-CD was basically nontoxic to HEK293 cells at the working concentration for pDNA delivery. Compared to unmodified PEI, PEI-β-CD induced nearly 4-fold higher luciferase expression [25]. By anchoring human insulin (which was derivatized with a hydrophobic palmitate group) onto its polyplexes, the transfection efficiency obtained could even be over an order of magnitude higher than that provided by unmodified PEI, either with or without the derivatized insulin [25]. Notwithstanding the prospects discussed above, it is worth noticing that the enhancing effect of CDs on PEI is valid only under the premise of proper optimization of the grafting ratio of CDs. This was revealed by the observation that modification of 5%, 10% and 16% of the amine groups in PEI with CDs reduced the luciferase activity by 1, 2 and 4 orders of magnitude, respectively [26]. This reduction was hypothesized to be due to the altered pK profile of the PEI amines, resulting in a decrease in the efficiency of endosomal release. Such a hypothesis was supported by the evidence that compared to unmodified PEI, PEI-β-CD exhibited a lower buffering capacity [26].

Another example of CD-linked polymers is linear βCDPs, which were synthesized from difunctionalized CDs and difunctionalized comonomers [47]. Similar to PEI-β-CD, high efficiency of this type of polymer in gene delivery necessitates fine structural optimization. Results of the luciferase activity assay in BHK-21 cells showed that the highest transfection efficiency was achieved by the linear βCDP with 6 methylene units [30]. The transfection efficiency of
linear βCDPs with 5, 4, 7, 8, 10 methylene units were only 6, 22, 50, 64 and 10% of that achieved by the one with 6 methylene units, respectively [30]. These results evidenced that different levels of CD incorporation can influence the transfection efficiency of the polymer [30]. Besides forming linear polymers, CDs have been used to fabricate star-shaped vectors, in which CDs function as the cores and other polymers as the arms. Examples of vectors formed by this approach are listed in Table 3 [48–52]. These vectors can facilitate pDNA delivery at different levels, and are worth further development for possible use in practical situations.

Apart from native CDs, derivatives of CDs have been used as linking agents. Previously, Huang et al. cross-linked PEI by using (2-hydroxypropyl)-β-CD (2-hy-β-CD) and (2-hydroxypropyl)-γ-CD (2-hy-γ-CD) [53]. The two resulting polymers exhibited lower cytotoxicity than PEI 25 kDa, and had transfection efficiency in SKOV-3 cells approximately 20 and 2 times higher than that achieved by PEI 600Da and PEI 25 kDa, respectively. More recently, β-CD has also been converted into the carboxymethyl-β-CD sodium salt, which has been combined with quaternized chitosan to form a DNA carrier. The carrier could not only adsorb pDNA perfectly at a polymer/DNA mass ratio of 4:1, but could also reach 40% of the transfection efficiency of the PAMAM control (G4, with an ethylenediamine core) in human neuroblastoma SH-SYSY cells. The polymer could not only adsorb pDNA perfectly at a polymer/DNA mass ratio of 4:1, but could also reach 40% of the transfection efficiency of the PAMAM control (G4, with an ethylenediamine core) in human neuroblastoma SH-SYSY cells.

Another example is transferrin, an iron-binding and -transport protein which functions as a targeting moiety towards various cancer cell lines (including those of colon cancer, ovarian cancer and glioblastoma) [56]. In a previous study, transferrin was conjugated to the poly(ethylene glycol) (PEG)-adamantane (PEG-AD) conjugate, which was subsequently incorporated into DNA nanoparticles of the linear imidazole-conjugated βCDP [57]. The transferrin-PEG-AD conjugate could not only self-assemble with the nanoparticles via inclusion complex formation between adamantane and the CD moieties on the particle surface, but could also retain high receptor binding activities. Luciferase activity assays in K562 leukemia cells found that the transfection efficiency of the nanoparticles surface-modified with transferrin-PEG-AD conjugates was 4-fold higher than that of the unmodified counterparts [57]. These results reveal the promise of ligand conjugation in enhancing the performance of polymeric vectors in gene transfer.

3.2. Functioning as structural modifiers

Aside from functioning as linking agents, CDs can be used to structurally modify existing gene carriers. Here CDs are utilized in two ways. The first is as threading devices. This is exemplified by the works from Li’s group, which has fabricated a range of supramolecular polyprotaxanes consisting of cationic α-CD rings threaded and blocked on a poly[(ethylene oxide)-ran-(propylene oxide)] (PEO-r-PO) random copolymer [58]. Approximately 12 α-CD rings were found in each molecule of P(EO-r-PO), with the rings being located selectively on EO segments of the copolymer. In HEK293 cells, the polyprotaxanes fabricated showed higher transfection efficiency than PEI 25 kDa [58], and deserve further evaluation as gene carriers for both in vitro and in vivo applications. The second way of utilizing CDs is as pendants. An example of vectors developed by this approach is the polyamidoamine (PAMAM) dendrimer conjugates with α-, β-, and γ-CDs. The conjugates could condense

| Table 3 | Examples of CD-based polymers with a star-shaped architecture. |
|---------|----------------------------------------------------------|
| Polymer | Description | Ref. |
| A star-shaped polymer consisting of a β-CD core and polyamidoamine (PAMAM) dendron arms | The polymer showed more than 1-fold higher transfection efficiency, but lower cytotoxicity, than the PAMAM control (G4, with an ethylenediamine core) in human neuroblastoma SH-SYSY cells. | [48] |
| A star-shaped polymer consisting of a γ-CD core and folate (FA)-modified oligoethylenimine (OEI) arms | The polymer exhibited low cytotoxicity, and demonstrated the ability to target and deliver DNA to specific tumor cells which over-expressed folate receptors (FRs). In addition, the polymer was reported to be able to recover and recycle FRs onto cellular membranes. This can facilitate continuous FR-mediated endocytosis of the polyplexes. | [49] |
| A CD derivative containing poly(-lysine) (PLL) dendrons | The derivative was prepared by click conjugation of per-6-azido-β-CD with the propargyl focal point PLL dendron. It could not only load methotrexate drugs and show a sustained release behavior, but could also complex with pDNA for transfection. | [50] |
| A star-shaped polymer consisting of a β-CD core and poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) arms | The polymer showed much lower cytotoxicity but higher transfection efficiency than high molecular weight PDMAEMA homopolymers. | [51] |
| A star-shaped polymer consisting of a β-CD core and poly(ethylene glycol)ethyl ether methacrylate)-modified PDMAEMA arms | Compared to the polymer consisting of a β-CD core and unmodified PDMAEMA arms, this polymer demonstrated higher transfection efficiency. | [51] |
| A star-shaped polymer consisting of an α-CD core and OEI arms | At an N/P ratio of 8 or higher, the polymer complexed with DNA to form polyplexes with a diameter of 100–200 nm. It gave transfection efficiency comparable to, or even higher than, that of PEI 25 kDa in HEK293 and Cos7 cells, but its cytotoxicity was significantly lower. | [52] |
A PAMAM starburst dendrimer conjugate with either \(\alpha\)-CD or \(\gamma\)-CD

The polypeudorotaxane allowed sustained release of pDNA. Upon intramuscular injection into mice, the transfection efficiency of the one with \(\gamma\)-CD lasted for at least 14 days.

\[69\]

A polyrotaxane with \(\beta\)-CD and \(\alpha\)-CD rings threaded onto ionone-6,10

The polyrotaxane formed stable complexes with pDNA and with a pDNA/siRNA mixture. It enhanced cellular uptake of the nucleic acid, and demonstrated low cytotoxicity.

\[70\]

A PAMAM starburst dendrimer conjugate with 6-O-\(\alpha\)-(4-O-\(\alpha\)-D-glucuronyl)-D-glucosyl-\(\beta\)-CD

The transfection efficiency of the conjugate was significantly higher than that of the unmodified \(\alpha\)- and \(\beta\)-CDE conjugates in A549 and RAW264.7 cells. It exhibited higher endosomal escape efficiency, and successfully delivered the transgene to the nucleus 6 h after transfection in A549 cells. 12 h after intravenous administration to mice, this conjugate provided higher gene transfer activity in the kidney than unmodified \(\alpha\)- and \(\beta\)-CDE conjugates.

\[71,72\]

A PAMAM starburst dendrimer conjugate with lactose-bearing \(\gamma\)-CD

In HepG2 cells, the conjugate exhibited higher transfection efficiency than unmodified PAMAM, lactosylated PAMAM and \(\beta\)-CDE. It also showed negligible cytotoxicity even up to a carrier/DNA charge ratio of 150/1. Compared to jetPEI\textsuperscript{TM}-Hepatocyte, the conjugate demonstrated higher transfection efficiency in hepatocytes 12 h after intravenous administration to mice.

\[73\]

\(\beta\)-CD-modified hyperbranched PAMAM

The polymer was fabricated by Michael addition copolymerization of N,N'-methylene bisacrylamide with 1-\(\beta\)-(2-aminoethyl)piperazine and mono-6-deoxy-6-ethylenediamino-\(\beta\)-CD. It demonstrated an ability to condense and deliver DNA.

\[74\]

A polypeudorotaxane with \(\gamma\)-CD rings threaded onto linear PEI

Compared to unmodified linear PEI, the polypeudorotaxane was more efficient in facilitating cellular uptake of pDNA in NIH/3T3 cells, and displayed much lower cytotoxicity.

\[75\]

\(\beta\)-CD-conjugated poly(\(\epsilon\)-lysine)

In NIH-3T3 cells, the transfection efficiency of the polymer was four orders of magnitude higher than that of unmodified poly(\(\epsilon\)-lysine), and was 10 times higher than that of linear PEI.

\[76\]

Table 4: Examples of polymers modified with CDs for gene delivery.

| Polymer | Description | Ref. |
|---------|-------------|-----|
| A polypeudorotaxane of the PEG-grafted \(\alpha\)-CD/PAMAM dendrimer conjugate | The polypeudorotaxane allowed sustained release of pDNA. Upon intramuscular injection into mice, the transfection efficiency of the one with \(\gamma\)-CD lasted for at least 14 days. | [69] |
| A polyrotaxane with \(\beta\)-CD and \(\alpha\)-CD rings threaded onto ionone-6,10 | The polyrotaxane formed stable complexes with pDNA and with a pDNA/siRNA mixture. It enhanced cellular uptake of the nucleic acid, and demonstrated low cytotoxicity. | [70] |
| A PAMAM starburst dendrimer conjugate with 6-O-\(\alpha\)-(4-O-\(\alpha\)-D-glucuronyl)-D-glucosyl-\(\beta\)-CD | The transfection efficiency of the conjugate was significantly higher than that of the unmodified \(\alpha\)- and \(\beta\)-CDE conjugates in A549 and RAW264.7 cells. It exhibited higher endosomal escape efficiency, and successfully delivered the transgene to the nucleus 6 h after transfection in A549 cells. 12 h after intravenous administration to mice, this conjugate provided higher gene transfer activity in the kidney than unmodified \(\alpha\)- and \(\beta\)-CDE conjugates. | [71,72] |
| A PAMAM starburst dendrimer conjugate with lactose-bearing \(\gamma\)-CD | In HepG2 cells, the conjugate exhibited higher transfection efficiency than unmodified PAMAM, lactosylated PAMAM and \(\beta\)-CDE. It also showed negligible cytotoxicity even up to a carrier/DNA charge ratio of 150/1. Compared to jetPEI\textsuperscript{TM}-Hepatocyte, the conjugate demonstrated higher transfection efficiency in hepatocytes 12 h after intravenous administration to mice. | [73] |
| \(\beta\)-CD-modified hyperbranched PAMAM | The polymer was fabricated by Michael addition copolymerization of N,N'-methylene bisacrylamide with 1-\(\beta\)-(2-aminoethyl)piperazine and mono-6-deoxy-6-ethylenediamino-\(\beta\)-CD. It demonstrated an ability to condense and deliver DNA. | [74] |
| A polypeudorotaxane with \(\gamma\)-CD rings threaded onto linear PEI | Compared to unmodified linear PEI, the polypeudorotaxane was more efficient in facilitating cellular uptake of pDNA in NIH/3T3 cells, and displayed much lower cytotoxicity. | [75] |
| \(\beta\)-CD-conjugated poly(\(\epsilon\)-lysine) | In NIH-3T3 cells, the transfection efficiency of the polymer was four orders of magnitude higher than that of unmodified poly(\(\epsilon\)-lysine), and was 10 times higher than that of linear PEI. | [76] |

pDNA and protect it from DNase I-mediated degradation [59]. In vitro studies showed that the \(\alpha\)-CDE conjugate (which is a dendrimer conjugate with \(\alpha\)-CD) had higher transfection efficiency than conjugates with \(\beta\)- and \(\gamma\)-CDs [59]. Its transfection efficiency in NIH3T3 and RAW264.7 cells was superior to Lipofectin, and was 100-fold higher than that of the unmodified dendrimer [59].

To boost the efficiency of gene delivery, \(\alpha\)-CDE prepared from the G2 dendrimer was galactosylated with various degrees of substitution (DS). Compared to the unmodified counterpart, the galactosylated conjugate with a DS value of 4 exhibited higher transfection efficiency in HepG2, NIH3T3 and A549 cells. Such an increase in transfection efficiency upon galactosylation, however, was found to be insensitive not only to the presence of competitors (asialofetuin and galactose) during transfection but also to the availability of asialoglycoprotein receptors on the cells to be transfected [60]. A similar phenomenon also happened in \(\alpha\)-CDE after mannosylation, which led to a receptor-independent increase in the efficiency of transfection [61]. The mechanism underlying this transfection enhancement is still unclear, but is proposed to be partially caused by the interaction between the modified conjugates and the intracellular galactose- or mannos-binding lectins [60]. Such an interaction was thought to have increased the efficiency of intracellular trafficking and nuclear translocation of the polyplexes [60]. In addition to ligand conjugation, the gene delivery efficiency of \(\alpha\)-CDE can be augmented by fine adjustment of structural parameters. For instance, compared to the \(\alpha\)-CDE conjugates synthesized from G2 and G4 dendrimers, the one constructed with the G3 dendrimer demonstrated higher transfection efficiency [62]. Furthermore, conjugates having different DS of \(\alpha\)-CD not only displayed different membrane-disruptive abilities on calcine-encapsulated liposomes [63], but also showed different cytotoxic activities and gene delivery capacities. In comparison with those having DS values of 1.1 and 5.4, the conjugate having a DS value of 2.4 showed higher transfection efficiency in NIH3T3 and HepG2 cells, and could deliver pDNA more efficiently to spleen, liver and kidney after intravenous administration [63]. These results point to the importance of structural optimization of the conjugate for transfection.

More recently, folate (FA)-appended \(\alpha\)-CDEs (FA-\(\alpha\)-CDEs) with various DS of FA have been synthesized from the G3 dendrimer [64]. As FA is known to have negligible toxicity, low immunogenicity and high affinity to FRs (whose isoform FR\(_A\) is over-expressed in cancer cells) [65–68], it is expected that after FA incorporation into \(\alpha\)-CDE, the resulting conjugate will exhibit higher tumor cell specificity and transfection efficiency. However, owing to the low receptor-binding activities of the resulting conjugate, no significant difference in transfection efficiency has been observed before and after FA incorporation [64].
To improve the FR-binding activity of FA-α-CDE, PEG has been used as a spacer between the dendrimer and FA, forming FA-PEG-α-CDE [64]. Among the three FA-PEG-α-CDEs (DS = 2, 5 or 7) fabricated, the one having a DS value of 5 performed the best in transfection, and demonstrated superior binding ability to both pDNA and FRs. 12 h after intratumoral injection into mice, FA-PEG-α-CDE (DS = 5) exhibited remarkably higher pDNA delivery efficiency than α-CDE. These results implicate the potential of FA-PEG-α-CDE (DS = 5) as a carrier for tumor-targeted gene delivery. Besides FA-PEG-α-CDE and other vectors that have been discussed in this section, there are many other polymers developed from modification of existing polymers using CDs. Some of them are listed in Table 4 [69–76].

These polymers have illustrated the potential of CDs in enhancing the performance of polymeric systems in transfection.

4. Implications for future research

In the sections above, we have delineated some of the major approaches of employing CDs for applications in gene delivery. These approaches are summarized in Fig. 3. For future research, one area that deserves exploration is drug/gene co-delivery. As CDs have an excellent drug loading capacity, along with their potential in gene transfer, CDs are anticipated to emerge as attractive candidates for simultaneous transport of drugs and genes.

![Fig. 3. Major approaches of employing CDs for applications in gene delivery. The arrows drawn with a broken line indicate that derivatives of CDs have not only been investigated as gene carriers, but have also been used as linking agents or structural modifiers.](image1)

![Fig. 4. Performance of TAT-PEI-β-CD in gene delivery to PMSCs. (A) Time course study of the transfection efficiency of TAT-PEI-β-CD and PEI-β-CD in PMSCs. (B) MTT assay in PMSCs after treatment with PEI 25 kDa, PEI 0.8 kDa, PEI-β-CD and TAT-PEI-β-CD. (Adapted from Ref. [80] with kind permission from Springer Science + Business Media B.V.).](image2)
unmodified hydrogel has been fabricated by radical copolymerization of PEG has been reported as a bifunctional anticancer prodrug [78].

The matrix was found to be able to transfect CCD fibroblast cells. [80].

At an N/P ratio of 20, the polymer displayed reasonable transfection efficiency in somatic cell lines (34–40% in Cho and HepG2, and 50–80% in 293T, U138 and U87). In placenta-derived mesenchymal stem cells (PMSCs), the level of transgene expression achieved by TAT-PEI-β-CD was comparable to that obtained by Fugene 6, and was approximately twice of that mediated by PEI-β-CD after 48 and 96 h of post-transfection incubation (Fig. 4A). Before and after transfection, the phenotypic profile of PMSCs was examined. Consistent expression profiles (including negativity for HLA-DR, CD45, CD38 and CD34, and positivity for CD147 and CD90) were observed. This suggests the maintenance of the phenotypic profile of PMSCs was cross-linked with bis-(2E)-adamantyljethyl)phosphatate. The matrix was found to be able to transfect CDE fibroblast cells.

Table 5

| Patent number | Year | Patent title | Details of the patent | Ref. |
|---------------|------|--------------|----------------------|------|
| EP 0762898 B1 | 1999 | Cycloextrin cellular delivery system for oligonucleotides | β-CD, or derivatives thereof, was used to facilitate intracellular concentration of the exogenous oligonucleotide was reported to be enhanced by using this strategy. | [94] |
| US 6022737 A | 2000 | Formulations for non-viral in vivo transfection in the lungs | β-CD was used as a component of a formulation for transfection. The formulation could enhance in vivo delivery of genes to the lung. | [95] |
| US 6509323 B1 | 2003 | Linear cycloextrin copolymers | Water-soluble, linear CD copolymers were synthesized as delivery vehicles of therapeutic agents. The copolymers were able to transfect BHK-21 and CHO–K1 cells. They could also deliver antisense oligos to inhibit the expression of the luciferase gene in HeLa X1/5 cells. | [96] |
| US 6884789 B2 | 2005 | Linear cycloextrin copolymers | | [97] |
| US 7091192 B1 | 2006 | Linear cycloextrin copolymers | | [98] |
| EP 1093469 B1 | 2007 | Linear cycloextrin copolymers | | [99] |
| EP 1764112 B1 | 2013 | Method for preparing linear cycloextrin copolymer | | [100] |
| US 8357377 B2 | 2013 | Cycloextrin-based materials, compositions and uses related thereto | A polymer composition was prepared using the following components: (i) a linear biocompatible polymer bearing inclusion hosts (such as CDs); (ii) linking molecules (each linking molecule comprised a PEG moiety and at least two adamantane moieties that formed inclusion complexes with the inclusion hosts); and (iii) at least one therapeutic agent which was covalently attached to the adamantane moiety. Complexes formed between DNA and the polymer composition were formulated with a matrix, in which CD-PEG was cross-linked with bis-(2E)-adamantyljethyl)phosphatate. The matrix was found to be able to transfect CDE fibroblast cells. | [101] |

Some patents on CD-based technologies for delivery of genes and other nucleic acids.

Apart from drug/gene co-delivery, the prospects of stem cell transplantation are also worth noting. Such prospects have been illustrated by a recent study, in which a cell-penetrating peptide containing the protein transduction domain (PTD) of the HIV-1 TAT protein has been conjugated to PEI-β-CD, forming TAT-PEI-β-CD [81–83], developing an efficient yet non-toxic and non-immunogenic gene carrier for stem cells will be of immense practical importance.

The last area that deserves future attention is small RNA transfer. Since advances in CD-mediated RNA delivery have been surveyed elsewhere [84], here we will not dwell on them. But it is worth pointing out that though the focus of our discussion in this article has been restricted to gene delivery, the possibility of a gene vector to interact electrostatically with DNA may imply that the same vector can complex with RNA [85]. This is confirmed by Arima et al., whose FA-PEG–CDEs have not only been found to mediate gene delivery [64], but have also been able to deliver siRNA to elicit RNA interference (RNAi) in tumor-bearing mice [86]. Detailed evaluation of the efficiency of each of the CD-based gene vectors will doubtless be needed if those systems are to be used for RNA transfer. Moreover, owing to the extra vulnerability of RNA to enzymatic degradation, additional challenges will be encountered when RNA rather
than DNA is to be delivered [85]. But regarding the emergence of RNAi and the bright prospects of RNA technologies in diverse areas, ranging from therapeutic target validation [87,88] to longevity enhancement [89], if existing CD-based gene delivery technologies turn out to be applicable to RNA transfer, not only can their medical applications be significantly broadened, but a vista of new opportunities will also be opened up for RNA-mediated therapies.

Finally, owing to the issue of intellectual property, innovations documented in the patent literature sometimes may not have been reported in scientific journals. Patent publications are thus a rich knowledge source complementary to the conventional scientific literature, and advances delineated in both scientific and patent publications should deserve the same amount of academic attention. The earliest patent on the pharmaceutical use of CDs can be dated back to 1953 [90]. Besides documenting the basic properties of CDs and disclosing a method to prepare CDs in aqueous solution via precipitation, the patent delineates the ability of CDs to improve the duration of activity, taste and chemical stability of bioactive compounds. The patent, however, has not been successfully put into industrial applications [91]. This is partially due to the safety concerns raised by a review article published in 1957 [92], which referred to unpublished data stating that rats fed orally with β-CD died within a week. Though the observed toxicity was later found to be more likely caused by impurities rather than the substance per se [93], the pharmaceutical applications and acceptance of CDs were hampered for many years and only until the 1970s the world first pharmaceutical formulation containing CDs emerged [13]. Now more and more innovations on CDs have appeared in the patent literature. Though at this moment most of these patents are related either to production and structural modification of CDs or to applications of CDs as drug excipients, some efforts have already been directed to the use of CDs in delivery of genes and other nucleic acids [94–101] (Table 5). Regarding the increasing awareness of the potential of CDs in gene delivery, it is expected that CD-based gene carriers will escalate in number in the patent literature in the forthcoming decades.

5. Concluding remarks

Gene delivery is an expanding area of biotechnological research, and has exhibited high potential in biomedical applications [102–105]. Though viral vectors entered clinical trials in as early as the 1990s and are still the most extensively studied gene delivery systems, the safety risks involved with using viruses warrant development of non-viral alternatives. Over decades, copious polymers have been investigated as gene carriers [106–111], but the possible use of CDs has rarely been seriously considered. This may pertain to the fact that native CDs fail to form stable complexes with pDNA [40], thereby showing much lower transfection efficiency in comparison with conventional polymeric vectors such as chitosan, poly(lactic-co-glycolic acid) (PLGA), PEI and PLL. However, judging from the evidence presented so far in this article, CDs have favorable properties for their applications in gene transfer (e.g. having the ability to form inclusion complexes with chemical drugs for drug/gene co-delivery, being able to potentially function as an absorption enhancer in therapeutics transfer, and being capable of modulating the cytoxicity of other polymers) and display great versatility in gene delivery, from functioning as linking agents for development of new vectors to modulating the performance of existing polymers in transfection. Taking the promising potential of CDs into account, there is no doubt that CDs will continue to emerge as an important tool for vector development, and will play significant roles in facilitating non-viral gene delivery in the future.

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