ChemistrySelect

Review
doi.org/10.1002/slct.202203004

Chemistry Europe
European Chemical Societies Publishing

www.chemistryselect.org

DNA Nanostructures in Pharmaceutical Applications

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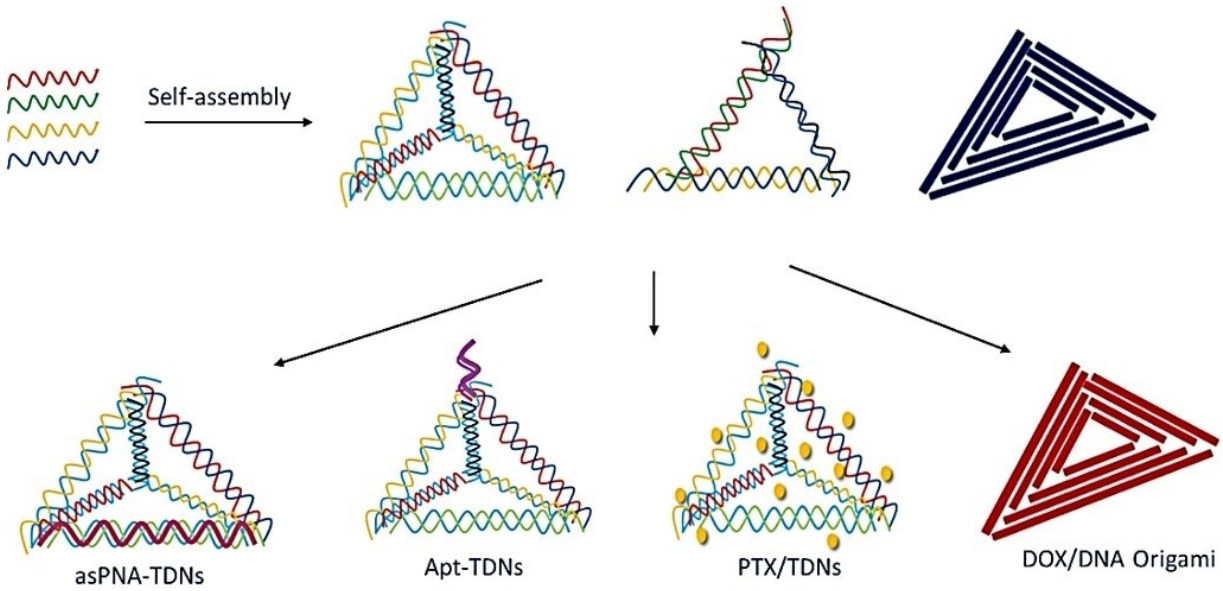

DNA nanostructures have evolved in various ways throughout the years since their discovery, including their structures and functions. Because DNA is a component of the biological system, it may be utilized to deliver therapeutic molecules without causing any harm to the body. For delivery, DNA nanostructures offer several benefits over conventional nanomaterials such as liposomes, polymers gold nanoparticles, carbon materials, etc. Various therapeutic molecules like drugs, nucleic acids, proteins, and a combination of these agents can be delivered with the help of DNA nanostructures.Because of the ease with which nanostructures can be synthesized through self-assembly and the ability to form a variety of shapes, they have found widespread application in biomedical fields. In this review, we will look at the different classes of compounds that are delivered via DNA nanostructures.

1. Introduction

Biomacromolecular components found in living organisms like nucleic acids include deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) which carry essential hereditary information and are associated with every form of life.[1] Over the years after the discovery of the DNA nanostructures by Nadrian C Seeman various developments have been made in the field and these advancements resulted in the emergence of various novel DNA nanostructures and their integrity and stability has been studied (Figure 1).[2–4] These nanostructures find applications in molecular computation, biosensing, and biomedical fields.[1]

In biomedical fields, different nanostructures including inorganic nanoparticles, micelles, and liposomes are used as drug delivery systems. But in comparison to other nanostructures, DNA nanostructures possess several advantages. Even though other nanostructures show high efficiency in delivery they possess potential toxicity and safety concerns.[5] The advantage of DNA nanostructures lies in their biocompatibility and negligible toxicity. They exhibit excellent programmability and addressability without compromising their stability.[1,5] By the manipulation of the Watson-Crick base pairing, a wide variety of DNA nanostructures with precise size and shape, number, positions, and functions of the functional groups are possible to create followed by the self-assembly process which is reproducible and predictable.[6,7] Different nanostructures such as DNA tetrahedron,[8,9] triangular DNA,[10] spherical nucleic acids,[11] and Y-shaped DNA[12] are used for the delivery of various therapeutic molecules.

In this review, we focus on how different DNA nanostructures could be used to deliver diverse therapeutic molecules such as drugs, nucleic acid, and proteins.

2. Small Molecules

Chemotherapeutic drugs have been successfully delivered via base intercalation as well as site-specific chemical conjugation utilizing chemically modified wireframe DNA nanostructures with fine control of shapes and sizes. To obtain a well-defined structural drug delivery system, these uniform DNA nanostructures can assemble drug molecules in a site-specific manner (Figure 2). The types of DNA nanostructures along with connected targeting components can be tuned to enable efficient cellular absorption. Meanwhile, regulated drug release behavior can be easily accomplished by embedding stimuli-responsive chemical bonds or nucleic acid motifs into chemically modified wireframe DNA nanostructures. Functional components that can be stimuli responsively cleaved or unfolded in certain conditions (such as reduction, oxidation, and acidification) and/or molecular triggers that can be targeted for effective regulated release are involved in these modifications.[13]

Tetrahedral DNA nanostructures are an excellent alternative vehicle for drug delivery and biomedical treatment.[1] Tetrahedral DNA nanostructures (TDNs) have shown high drug loading and sustained drug release capabilities among these DNA nanostructures. Chemotherapeutic drugs, gene therapy agents, active protein, and imaging probes can all be easily integrated into these TDNs and given to tumor sites through the increased drug diffusion and retention (EPR) effect. Functional moieties (such as targeting aptamers, peptides, and antibodies) can also be carefully painted on TDN to enable targeting.[14] (Table 1)

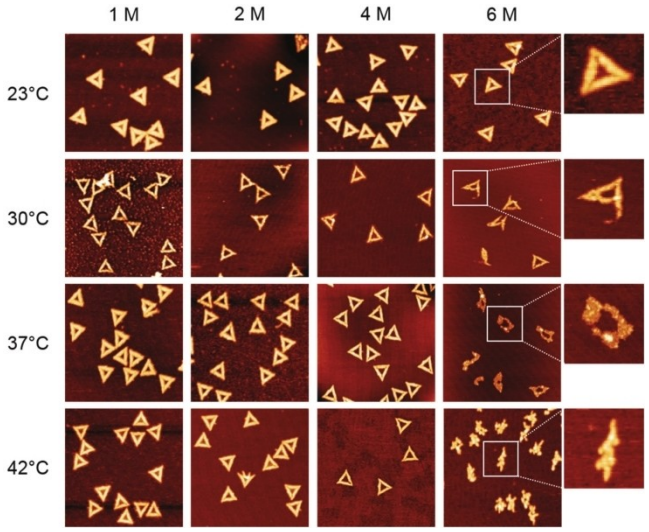

Figure 1. AFM images of DNA origami triangle after 1hr incubation in urea at different concentrations and temperatures. Reproduced from ref.[3], Copyright (2016), with permission from Royal Society of Chemistry.

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2.1. Fluorescein Dye

Ding and co-workers recently reported on a label-free fluorescence approach for studying the distribution and stability of DNA origami nanostructures in living cells. Carbazole-based Biscyanine, a type of dye, could attach to a DNA duplex and emit intense fluorescence in a compact DNA structure. When the compact DNA structure was disturbed, however, the fluorescence intensity decreased. They used DNA origami structures to transport carbazole-based biscyanine into cells and discovered that this nanocarrier could withstand degradation for at least 60 hours in cells, demonstrating that it might be used to control cargo release.[15]

Mao and co-workers introduced DNA nanotubes conjugated folate (a molecule targeted to cancer cells) and fluorescent dye Cy3 which showed no obvious toxicity to living cells.They also demonstrated great targeting efficacy in cancer cells overexpressing the folate receptor, delivering the binding fluorescent molecules into the cell without the use of transfecting reagents. The minimal immunogenicity of bare DNA

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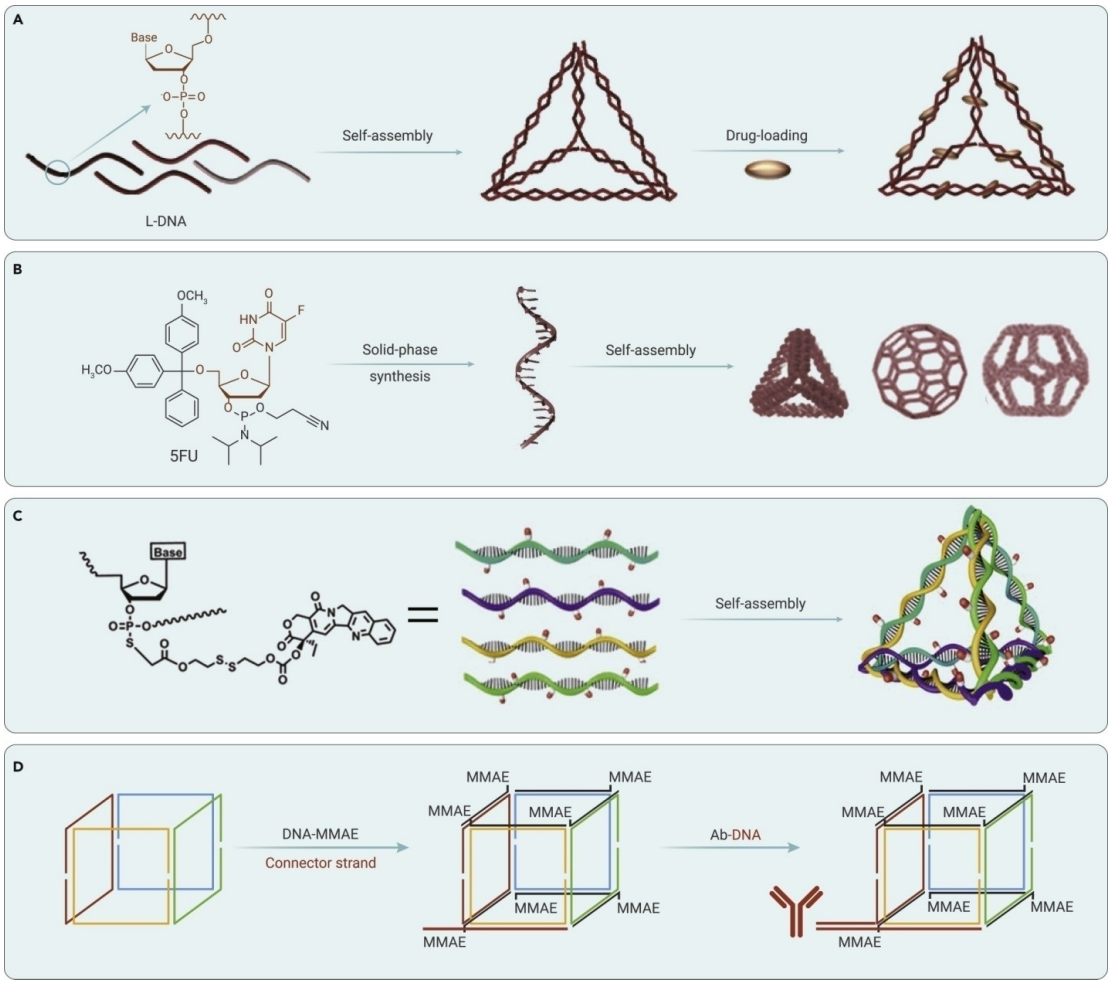

Figure 2. Chemotherapeutic drug delivery using chemically modified wireframe DNA nanostructures (A) Mirror DNA tetrahedron for tumor-specific DOX delivery. (B) DNA polyhedral containing fluorouridine for cancer therapy. (C) Camptothecin-grafted DNA tetrahedron for cancer therapy. (D) Antibody conjugated Wireframe DNA Cube for targeted delivery of MMAE. Reproduced from ref.[13], Copyright (2022), with permission from Elsevier.

Table 1. Various drugs delivered by DNA nanostructures and the advantage of DNA nanostructures.

Small Molecules	DNA nanostructure non-enabled	DNA nanostructure enabled
Fluorescein Dye[15,16]	Low stability Low endurance of fluorescence intensity	Higher stability Higher endurance of fluorescence intensity Withstand degradation for at least 60 hrs in cells
Doxorubicin[13,15,17–20]	Negative side effects Poor selectivity	Improve efficacy Reduce adverse effects Overcome drug resistance
Monomethyl Auristatin E[13]	Variances occur in a drug-to-antibody ratio	Precision is achieved with a stable drug-to-antibody ratio
Paclitaxel[21]	Low efficacy Hydrophobicity Multidrug resistance	Greater drug accumulation Relatively high efficacy Reduction of drug resistance

nanostructures was demonstrated in research on DNA cages or origami-immune stimuli hybrid nanostructures, demonstrating the biosafety of DNA materials.[16]

2.2. Doxorubicin (DOX)

Doxorubicin (DOX) is a powerful anti-cancer medicine that works by intercalating the DNA duplex and blocking macromolecular formation to treat a variety of malignancies. It is, however, well known for having negative side effects and poor selectivity. To improve therapeutic efficacy, reduce adverse

effects, and overcome drug resistance, many delivery carriers have been created, including polymer micelles, liposomes, nanoparticles, and inorganic nanomaterials. DOX, for example, can non-covalently bind to double-stranded DNAs by intercalation into the helix, which can be used to transport DOX via DNA structures.[17]

Kim et al. created a mirror DNA tetrahedron for the loading of the chemotherapeutic medication doxorubicin by co-assembling four single-stranded L-DNA which were predesigned in 2016. The serum stability of the mirror DNA tetrahedron was higher than that of the regular D-DNA tetrahedron. The increased therapeutic effects in vivo were due to the longer stay duration of the mirror DNA tetrahedron in the tumor site. In addition, the same group created a library of mirror DNA nanostructures of various shapes and sizes to load DOX for drug delivery in 2019. They discovered that the size and form of chemically modified DNA nanostructures must be carefully designed to achieve efficient cellular absorption and macrophage evasion. Ultimately, a pyramid mirror DNA cage structure exhibited the greatest tumor specificity and brought in an effective therapeutic effect.[13]

DNA origami nanostructures were utilized by Wiraja et al. to deliver DOX for the treatment of melanoma in vivo using a transdermal drug delivery approach. When compared to topically applied free DOX or DOX loaded in liposomes and polymeric NPs, the DOX-loaded DNA-nanostructures produced a more than two-fold improvement in drug accumulation and tumor growth suppression.[18]

It was discovered that the DNA origami dual imaging and drug delivery system showed optimal anticancer efficacy in vivo with no systemic toxicity when QD655-labeled triangular DNA origami structures loaded with DOX were given to tumor-bearing mice and tumor growth and antitumor efficacy of drug-loaded DNA carriers were observed using non-invasive fluorescence imaging.[18]

Tetrahedral DNA nanostructures (TDNs) were used in a study to enable the mitochondrial transportation of the anticancer medication doxorubicin (DOX) for cancer therapy, and it was discovered that DOX was intercalated into TDNs, which carried out the cell-killing function inside tumor cells.[14]

Ding and co-workers have published a DOX delivery device based on DNA origami nanostructures. To load DOX, they used two-dimensional triangle DNA origami and three-dimensional DNA tubes, which showed a higher loading efficiency than expected based on calculations and were partially confirmed by electrophoresis gels and AFM pictures. The significant enhancement of cell killing activity in doxorubicin-resistant MCF 7 cells was discovered after the DNA nanostructure-doxorubicin complexes were administered to regular

human breast adenocarcinoma cancer cells MCF 7 (reg-MCF 7) and a cell subline that is doxorubicin-resistant (res-MCF 7). This was due to the prominent cytotoxicity and increased internalization of doxorubicin. Based on their findings, they also assumed that the activity of doxorubicin-loaded DNA origami hindered lysosomal acidification, which resulted in drug redistribution to action locations in the cell.[15]

DNA origami nanostructures were able to deliver DOX to three different breast cancer cell lines, according to Hogberg et al. They created straight and twisted DNA origami tubes using a honeycomb lattice framework. The twisted DNA origami tubes for DOX have a higher loading capacity, and a slower release rate, and are more stable due to the different degrees of relaxation in the DNA double helix structure. As a result, they claimed that altering the nanostructure might control drug release kinetics and encapsulation efficiency. When collated to free DOX, their findings demonstrated that these DNA nanostructures aided to boost cytotoxicity and lower intracellular clearance rate.[15]

Recently, a DNA origami-based drug delivery system was developed by Jiang et al, which had a high DOX loading efficiency. Not only human breast adenocarcinoma cancer cells (MCF-7) but also doxorubicin-resistant cancer cells displayed the desired cytotoxicity with this complex. In the meantime, DNA origami carriers improved the cellular internalization of DOX, boosting the cell-killing action of DOX-resistant MCF-7 cells significantly.[19]

Zhao et al created two DNA origami nanostructures to deliver DOX to three breast cancer cell lines. DOX was intercalated in the DNA double-helix structure of these two nanostructures with varying degrees of global twist. The drug encapsulation efficiency and drug release kinetics could be controlled by modifying the nanostructures. The cytotoxicity of Dox given by these two nanostructures was higher than that of free DOX, while the rate of intracellular rate was decreased.[19]

Yang et al used a hydrogel/nanosystem to create a chemo-assisted immunotherapy system that included DC and a pH-sensitive immune adjuvant DOX nano-prodrug. The pH-sensitive nano-prodrug system ameliorated the controlled release of DOX while lowering its negative effects.[20]

2.3. Monomethyl Auristatin E (MMAE)

Märcher et al. structured an antibody onto a chemotherapeutic drug monomethyl auristatin E (MMAE, a tubulin inhibitor)-loaded wireframe DNA cube in 2021 for target delivery of a drug-conjugated wireframe DNA nanostructure. A cleavable organic linker was used to covalently attach the MMAEs to single-stranded DNA. Through effective DNA hybridization, the single-stranded DNA-conjugated antibody was successfully linked to the remaining vertex of the seven MMAE-loaded DNA cubes. The drug-to-antibody ratio is precisely set to 7:1 in this situation. In classic antibody-drug conjugates, such precision is often impossible to achieve. In vitro, the antibody (trastuzumab)-DNA cube-MMAE was found to be an effective target in HER2-positive SKBR3 cells. The use of uniformed DNA nanostructures as a template for the site-specific loading and targeting groups of chemotherapeutic medicines is demonstrated in this report.[13]

2.4. Paclitaxel (PTX)

Paclitaxel (PTX) which is a kind of plant-based antineoplastic drug, impedes cell division by inhibiting mitosis and finally

results in cell apoptosis.[19] Paclitaxel (PTX) is a drug that is effective against various cancers which include ovarian cancer, breast cancer, and ovarian cancer. Even though multidrug resistance restricts the clinical applications of PTX, Tetrahedral DNA nanostructures (TDNs) can act as a magnificent drug delivery candidate.[21]

It's used to treat a variety of malignancies, including ovarian and non-small cell lung tumors, but its widespread use is hampered by low efficacy, hydrophobicity, and multidrug resistance. As a result, PTX-DNs, which enable drug resistance reversal and strong cytotoxicity, has become a hot topic in recent research. The drug molecules can be transported into the cytoplasm and released by loading PTX with TDNs, resulting in greater drug accumulation and relatively high efficacy, as well as the reduction of drug resistance.[21]

In a study, TDNs loaded with PTX (PTX/TDNs) were used and the cytotoxicity of PTX/TDNs and PTX alone on non-small cell lung cancer (NSCLC) cells (A549) and the PTX resistant cell line (A549/T) was determined and it was found out that PTX/TDNs wielded strong lethality on both cell lines and the drug resistance was overcome and the study also showed that PTX/TDNs destroyed cancer cells via apoptosis. As a result, PTX/TDNs offers a lot of potential as a nano delivery method for treating PTX-resistant NSCLC. Because of the relatively efficient internalization of PTX by the drug system, studies have shown that PTX/TDNs can impede the proliferation of both multidrug-resistant and wild-type cells.[21]

2.5. 5-Fluorouracil (5-FU)

5-fluorouracil is a widely used antineoplastic agent primarily used to treat colorectal and breast cancers and its lack of target specificity is a major limitation of its application. As a novel tumor targeting nanomedicine, Zhan et al. created a DNA tetrahedron with a DNA aptamer (AS1411) delivery system attached with 5-FU (AS1411-T-5-FU). The results of the study showed that the AS1411-T-5-FU possessed biocompatibility, stability, and preferential killing ability toward the tumor cells. AS1411-T-5-FU also demonstrated improved therapeutic efficiency against breast tumor cells due to greater absorbability than 5-FU alone. The findings showed that AS1411-T-5-FU has the potential to be developed as a novel strategy for treating malignant tumors.[22] In addition to these drugs, some other anticancer nucleoside analogs could be introduced to the DNA nanostructures including 6-mercaptopurine.[23]

3. Nucleic acids

Gene therapy is a clinical procedure that involves the use of therapeutic nucleic acids or gene regulators to treat diseases. The therapeutic drugs include antisense oligonucleotides (ASOs), small interfering RNA (siRNA), DNAzyme, or a whole gene editing system. Immunotherapy is another method of treating various diseases which are categorized into active and adoptive immunotherapy. This category includes Cytosine-Phosphate-Guanosine (CpG) oligodeoxynucleotides and aptamers. One of the major issues in developing a nonviral vector that can deliver the nucleic acids to the target is to which the DNA nanostructures provide an excellent solution(Table 2).[21] The different therapeutic agents can be introduced to the DNA nanostructures by base-pairing hybridization or by chemical covalent cross-linking (Figure 3).[13]

3.1. CpG oligodeoxynucleotides

Unmethylated DNA molecules that contain a cytosine(C) triphosphate deoxynucleotide connected to guanine (G) triphosphate deoxynucleotides by phosphodiesters(p) is known as CpG oligodeoxynucleotides. ImmunostimulatoryCpG ODNs are a special class of therapeutic drugs.[24] These CpG are found on bacterial and viral DNA, when they are exposed during infection, they trigger the Toll-like receptor(TLR), which triggers a powerful immune response.[6] CpG ODN is delivered using a variety of DNA nanostructures, including DNA tetrahedrons, DNA origami, and so on. However, several of them had limits. Qu et al. demonstrated that DNA microcapsules are an effective method for delivering CpG ODNS. They used hybridization to create a Y-shaped CpG from three single strands. It stayed as a single strand and could be used for other base pairings. After that, it was injected into porous $CaCO_3$ particles. To these Y-CpG capsules, the duplex DNA linker with complementary sticky ends was added. The $CaCO_3$ was dissolved, resulting in

Table 2. Different nucleic acid drugs are delivered by various DNA nanostructures.

Nucleic acid drugs	DNA Nanostructure	Therapy
CpG ODNs	DNA microcapsules[12]	Immunotherapy
siRNA	DNA tetrahedron[27]	Gene therapy
	DNA nanogel[28]	
DNAzyme	SNA[10]	Gene therapy
	DNA tetrahedron[29]	
Aptamers	FNA[31]	Immunotherapy
	DNA tetrahedron[1]	
miRNA	DNA tetrahedron[33,34]	Gene Therapy
	Nanogel[35]	
ASOs	SNA[36]	Gene Therapy
mRNA	SNA like nanogel[37]	Gene Therapy
	EVs with DNA aptamer[38]	
	DNA nanoclew[39]	
	Nanogel[40]	

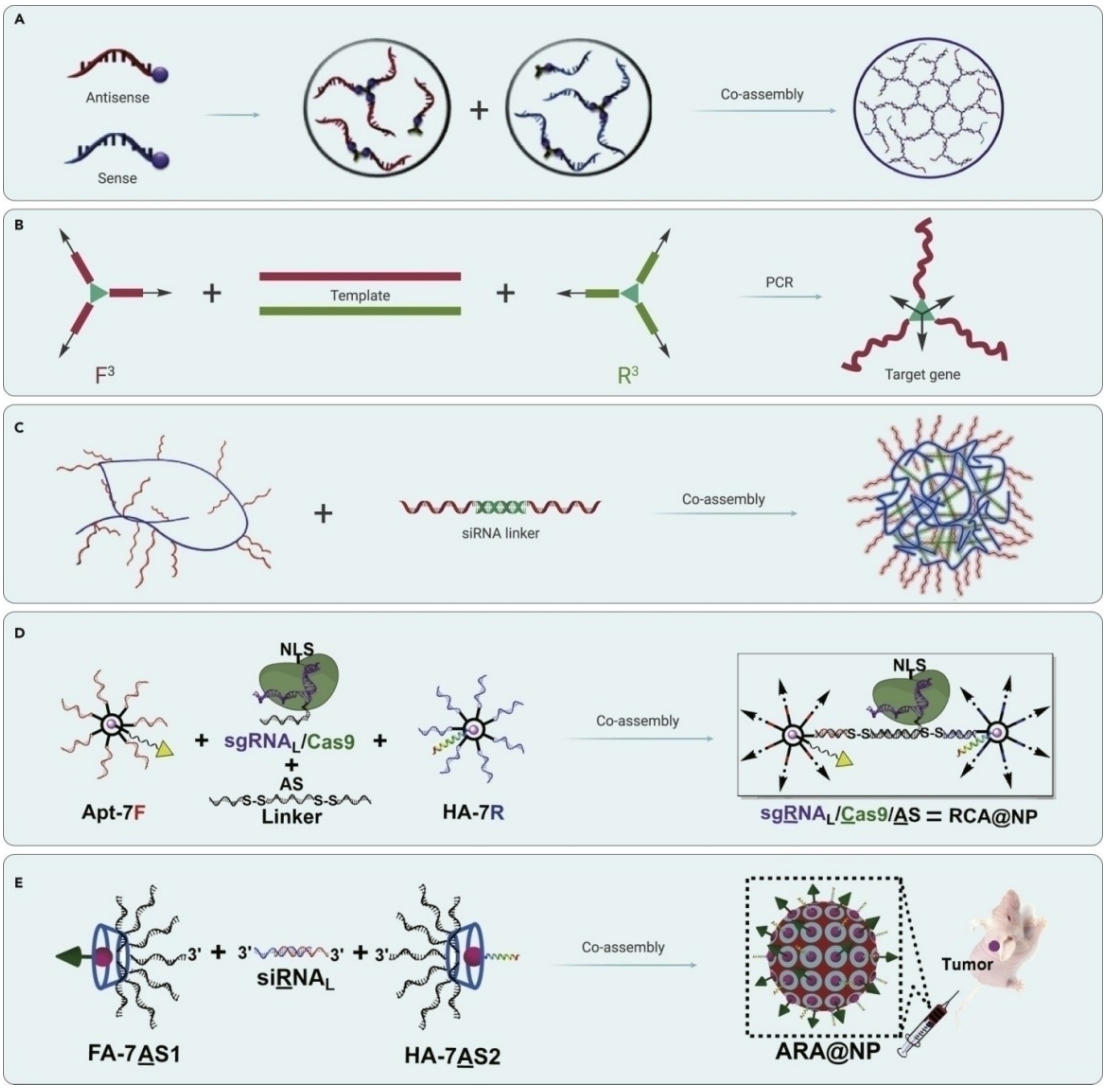

Figure 3. Delivery of nucleic acid drugs based on chemically modified nucleic acid nanostructures (A) Silencing of gene using siRNAmicrohydrogels. (B) Stable gene nanoparticles constructed by branched PCR for gene delivery. (C) Crosslinked nucleic acid nanogel for siRNA delivery. (D) Branched DNA for delivery of sgRNA/Cas9/antisense .(E) Branched antisense and siRNA co-assembled nanoparticle for combined gene silencing. Reproduced from ref.[13], Copyright (2022), with permission from Elsevier.

DNA microcapsules (Figure 4). DNA capsules containing CpG ODNs have shown to be effective as both a gene delivery vehicle and an immunomodulator.[12]

3.2. Small interfering RNA (siRNA)

Small interfering RNA(siRNA) is a type of double-stranded noncoding RNA that identifies and cleaves complementary mRNA.[25] The down-regulated mRNA induced to degrade protein expression. Different nanocarriers, similar to CpG, can be utilized to carry siRNA.[26] Wireframe nucleic acid nanostructures which are formed by the self-assembly of multiple oligonucleotides are a promising platform for drug delivery. They are useful in delivering ASOs, siRNA, and aptamers and they can be loaded easily by base pairing. Thai et al. for the delivery of siRNAs specifically to kidneys, developed small-sized DNA tetrahedrons (Figure 5). For the effective accumulation of the DNA tetrahedron in the kidney, they have to be small, but at the same time, they must also include enough base pairs for the stable assembly of the nanostructures. So they developed a tetrahedron with 10 base pairs on one side with different sugar backbones. Each of the tetrahedrons was characterized and the studies showed that L-sTD (small tetrahedron) has kidney-preferred biodistribution. The mRNA p53 was loaded to this L-sTD. The siRNA with a target-specific sequence was able to down-regulate the gene. Even though naked siRNA could be delivered into the cells they were unable to silence the gene. L-sTDS were proven to be effective for the delivery of siRNA and the recovery of kidney function in their studies.[27]

Even though DNA tetrahedron and spherical nucleic acids (SNA) can be used for the delivery of siRNA, there is a risk of degradation if the siRNA is exposed outside of the system.To

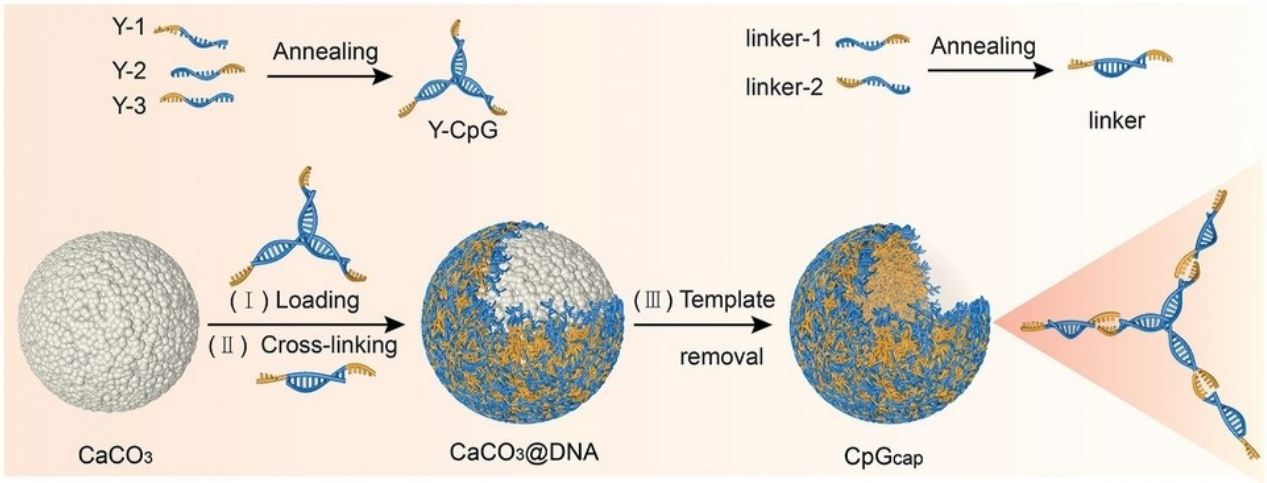

Figure 4. Schematic representation of the template-mediated assembly of CpGcap: I) loading of $CaCO_3$ particles with Y-CpG; II) cross-linking by the duplex DNA linker; and III) removal of $CaCO_3$ particles to yield CpGcap. The yellow strands on DNA represent sticky ends from the Y-CpG and linker, which are complementary to each other. Reproduced from ref.[12], Copyright (2020), with permission from Wiley-VCH.

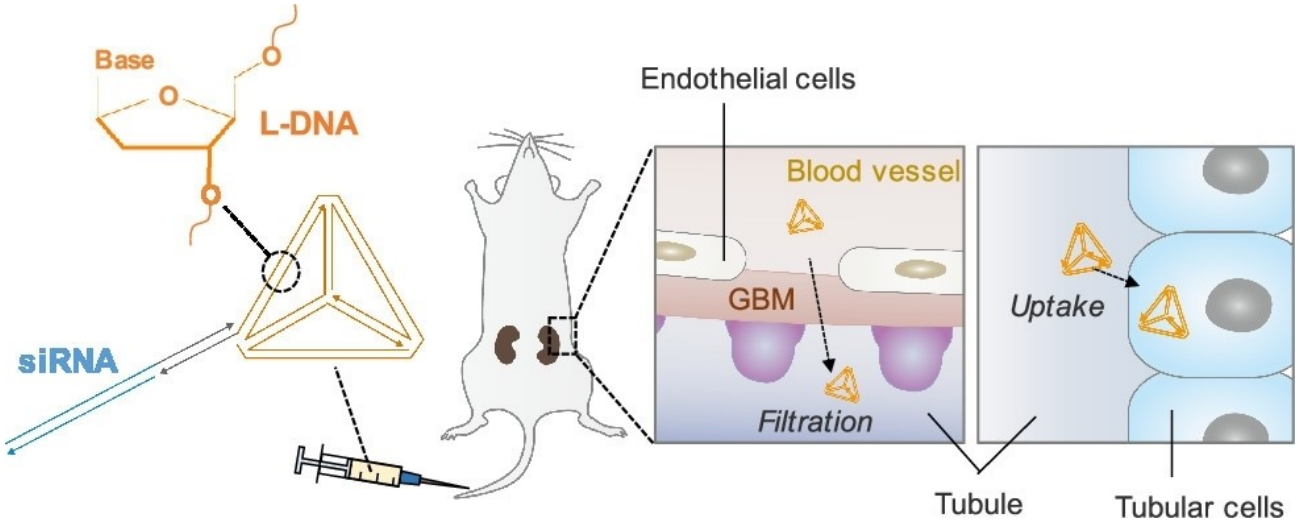

Figure 5. Schematic representation of delivery of siRNAs to kidneys using L-sTDs. Reproduced from ref.[27], Copyright (2020), with permission from American Chemical Society.

eliminate the risk, Xue et al. developed a DNA nanogel loaded with cross-linked siRNA. They attempted to use a DNA nanogel with DNA grafted polycaprolactone and siRNA as backbones and linkers respectively. But the synthesis requires laborious work so instead of DNA grafted polymer, the siRNA was embedded in a nanostructure and protected for cellular delivery. The nanogel thus synthesized showed good stability as it prevented the loaded siRNA from degradation. Also, the nanogel knocked down the gene expression without any transfection agents. The research showed that the nucleic acid-based nanogel system is an emerging platform for siRNA-based therapy that can also be employed for the delivery of other oligonucleotides.[28]

3.3. DNA Enzyme (DNAzyme)

DNA enzymes (DNAzyme) are single-stranded DNA that catalyzes specific reactions like the target DNA or RNA cleavage and ligation of DNA phosphorylation in combination with their cofactors.[11,26] SNA can be used as the delivery vehicle for DNAzyme, but they were uptaken by the cells. To prevent this and to provide good lysosome escape capacity of functional nucleic acids, aggregation-induced emission (AIE) photosensitizers can be utilized. Shi et al. synthesized a carrier-free DNAzyme delivery system with AIE photosensitizers and its corresponding cofactor Zn^{2+}. This hybrid DNAzyme could produce O_2 for the rupture of the lysosome and the nanostructure could effectively silence the target mRNA and prevent

the expression of the protein. The O_2 produced can also induce cell apoptosis in tumor cells.[8] Dz13 is a DNAzyme that cleaves the mRNA of c-Jun and suppresses the growth of carcinoma cells. DNA nanostructures can be used to deliver the Dz13 DNAzyme. Meng et al. developed a tetrahedral DNA nanostructure (TDN) to deliver the Dz13 sequence into the cells. TDN–Dz13 entered the cells and it demonstrated excellent gene silencing ability, thus decreasing the cell proliferation by c-Jun mRNA. The work showed the advantages of TDN as carriers and the various applications of DNAzyme.[29]

3.4. Aptamers

Aptamers are single-stranded DNA or RNA molecules that bind to their target with high affinity and specificity. Aptamers are widely conjugated to the surface of the drug carriers to enhance their targeting properties for therapeutic and diagnostic reasons. Furthermore, certain aptamers can activate downstream signaling pathways after binding to their receptors, allowing them to be employed as therapeutics.[30] Li et al. developed a bipyramidal framework of nucleic acids (FNA) for delivering anti-C5a aptamers. Anti-C5a aptamers have high affinity and specificity for C5a and they reduce the C5a-mediated inflammatory and neuronal damage. The studies showed that the DNA framework is an excellent antioxidant for reactive oxygen species in both solutions and on the cellular level to protect primary neurons from oxidative stress. The C5a-loaded FNA strongly binds to the C5a and can result in the inhibition of the chemotaxis of inflammatory cells in its response.[31] Zhang et al studied the use of TDN-based delivery complexes such as antisense peptide nucleic acid (asPNA) embedded TDNs, aptamer modified TDNs, etc. in the field of tissue generation and anticancer and antibacterial treatment. HApt is an aptamer with an affinity towards HER2 in breast cancer cells and the HApt-TDNs were observed intracellularly in HER2 positive cells showing the targeting ability of TDNs and the aptamer inducing cell apoptosis of HER2 positive cells. Similarly, PNA-TDNs inhibit bacterial growth in the log phase.[1]

3.5. Transcription Factor Oligonucleotides

Different studies have demonstrated that DNA nanostructures can be used to deliver nucleic acid drugs for the treatment of Rheumatoid arthritis (RA). There are artificial DNA that contains sequences that are specific to prevent the binding of transcription factors and regulate gene expression and are called transcription factor oligonucleotides.[30] Wang et al. studied the anti-inflammatory efficacy of engineered DNA nano drugs such as TD-dODN (DNA tetrahedron with Nf-κB decoy oligonucleotides) and TD–P-dODN (TD-dODN with a peptide). The nanodrugs with the dODNs and peptides were synthesized by self-assembly and these drugs showed increased stability compared to the free dODNs. The nano drugs exhibited reduced levels of inflammatory proteins and proved to be beneficial for the RA treatment by inhibiting the Nf-κB signaling pathway in the inflammatory cells.[32]

3.6. miRNA

Li et al. modified MiRNA-214-3p to complex with TDN and observed the ability of the miRNA (micro RNA) to induce tumor cell apoptosis. Using the TDNs the instability of miRNA can be overcome and thus their intracellular efficiency can be increased. Survivin is an inhibitor of apoptosis protein and is seen in malignancies more than in normal cells. MiRNA-214-3p can bind to the survivin mRNA and can induce tumor cell apoptosis. The work showed that the miRNA incorporated TDNs can induce cell apoptosis in tumor cells.[34] MiRNA-155 can down-regulate the expression of the inflammatory proteins in microglia and macrophages. The increase in the miR-155 levels can reprogramme the anti-inflammatory M2 type microglia and macrophages to pro-inflammatory M1 type. Gao et al. designed a nucleic acid nanogel with erythrocyte membrane coating, that mimick virus structure and can successfully deliver therapeutic miR-155 to the microglia and macrophages (Figure 6). The studies proved that the membrane coating improved the circulation lifetime of miRNA-155 and further modification in structure resulted in the active tumor targeting and tumor inhibition capability.[35]

The sensitive identification of tumor-related miRNA offers considerable potential for cancer diagnostics. Su et al. reported the first example of vertebral-shaped tetrahedral DNA nanostructures for miRNA silencing-induced therapy and cancer detection. TDNs were prepared using seven single-stranded nucleic acid chains and fluorophores were linked to their vertexes. These fluorophores were quenched with adjacent quenchers. Three intracellular miRNA expression levels could be quantitatively detected with high sensitivity and specificity based on the fluorescence “OFF” to “ON” mode.In the presence of target intracellular miRNAs (miRNA-21, miRNA-122, and miRNA-194) the complementary sequences were released from TDNs and hybridized with the target to form stable double strands. Here using the TDNs simultaneous detection of three miRNAs was possible and it reduced the chances of false positive signals. The TDNS also delivered antagomir-21 into cells thus silencing the miRNA-21 and inducing cancer cell apoptosis. the studies demonstrated that the TDNs effectively distinguished tumor cells from cancer cells and suppressed tumor growth by silencing miRNA-21.[33] Li et al created a biosensor using DNA for the detection of has-miR-21-5p with excellent sensitivity. The presence of the miRNA was determined in serums collected from different cancer patients and as well as treated patients.[41]

3.7. mRNA

CRISPR/Cas9-based gene-editing tools are more efficient gene editing platforms than TALENs and ZFNs as they target and edit the sgRNA sequence in the interested gene. Ding et al. proposed the use of noncationic SNA-like nucleic acid nanogel for the intracellular delivery of the Cas9/sgRNA complex, which protects the complex inside and rapidly enters the cells allowing effective target gene editing. The gene-editing tool showed great stability in the delivery platform but was able to

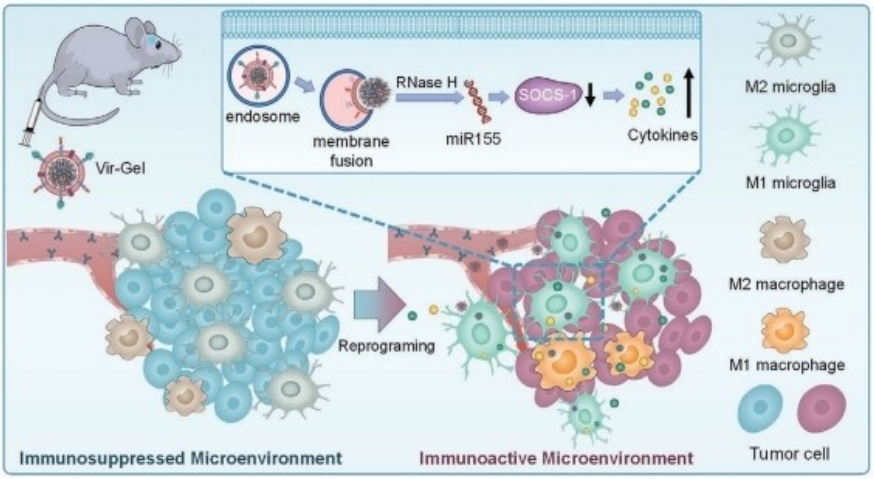

Figure 6. Schematic illustration of the virus mimicking nucleic acid nanogel (Vir-Gel).Vir-Gel shifts microglia and macrophage polarization from an anti-inflammatory M2-phenotype to a pro-inflammatory M1-phenotype bt miR155-mediated promotion of cytokine production. Reproduced from ref.[34], Copyright (2021), with permission from Wiley-VCH.

be released in the presence of a nuclease that stimulates the intracellular environment. The studies demonstrated the system could enable efficient gene editing in vitro.[37]

Zhuang et al. created extracellular vesicles(EVs) engineered with valency-controlled DNA nanostructures to deliver the CRISPR/Cas9 system. EVs are cell-derived membrane vesicles that are suitable for a cell to cell communication, but they show poor targeting ability. DNA aptamer can be conjugated to the EV surface to increase its targeting ability and TDNs were used to preciously anchor DNA aptamer to the EV surface. The studies showed that the loading process did not significantly alter the morphology and characteristics of native EVs. Thus Zhuang et al. developed a cell-specific exosomal delivery of CRISPR-Cas9 RNA-guided endonucleases (RNP) with DNA nanotechnology.[38]

Sun et al. developed a non-viral carrier for the hepatocyte-targeted delivery of the CRISPR-Cas12a system which reduces the serum level of cholesterol. They used a self-assembled DNA nanoclew to load CRISPR-Cas12a RNP to the target. The DNA nanoclew carrier provided an anionic core to load CRISPR-Cas12a RNP and also enabled the layer-by-layer deposition of cationic and charge reversal layers. The system demonstrated a highly biocompatible delivery system for CRISPR-Cas12a and the assembly obtained therapeutic effects by efficient gene disruption thus reducing cholesterol levels.[39]

A nucleic acid-based nanogel system was developed by Huang et al. for the efficient delivery of mRNA into the cells. DNA nanogel encapsulated the mRNA by hybridization of the noncoding poly(A) tail of mRNA with the DNA strands of the carrier. Further, the nano gels were coated with polyethylenimine (PEI) after the gel formation.The encapsulated mRNA was protected by the nanogel and the studies showed that the mRNA/nanogel/PEI-treated cells have high transfection efficiency compared to mRNA/PEI, mRNA alone, or mRNA/nanogel. They further extended the studies to demonstrate whether the nanogel was able to deliver mRNA encoded with functional proteins. The results demonstrated that the nanogel was able to deliver Cas9-mRNA efficiently to the target cells for the CRISPR-Cas9 genome editing. The studies proved the DNA nanogel system as an efficient delivery system for mRNA and mRNA encoded with therapeutic proteins to establish novel approaches in medicine.[40]

Liu et al. reported branched DNA-based nanoplatforms for the sgRNA/Cas9/Antisense delivery for synergistic therapy. The DNA nanostructures proved to be an efficient codelivery vehicle due to their efficiency in loading and release of the therapeutic cargoes. The studies demonstrated that the DNA platform could deliver additional therapeutic components including immune-related adjuvants and antibody-based drugs.[42]

3.8. Other Nucleic Acid Drugs

Dai et al. developed a triangular DNA nanostructure with DNA aptamer and ASOs that can enter the cancer cells without any transfection agent.[10] The aptamer served to target purposes whereas the ASO was able to down-regulate the gene expression at both mRNA and protein levels. The studies showed that the nanostructures with ASO sequence targeted at a specific site at the target gene and thus reduced the amount of the ASO drugs that need to be used. The results proved that the system is bio-orthogonal and safe for biomedical applications.[10] Mou et al. developed a two-in-one chemogene with ASO as the therapeutic drug as well as an agent to reverse the drug resistance in cancer cells. They synthesized a drug integrated ASO-based spherical nucleic acid nanostructures; formed by the self-assembly; which posses to have the ability of efficient cellular entry. The ASO-based delivery system proved to be an excellent tool for the delivery of drugs as well as, can act as therapeutic genes to reverse the

chemoresistance of the cells.[36] Liu et al. prepared a DNA nanodevice that contained peptides as the antigens and dsRNA (double-stranded RNA) and CpG motifs as adjuvants for cancer immunotherapy. The nanodevice was also incorporated with DNA locks that were pH-responsive. The locks bound to the rectangular DNA close the platform to a tubular nanostructure which shields the antigens and adjuvants inside. The delivery using the DNA platform showed a strong tumor-specific CTL response and efficient tumor inhibition and regression. It is beneficial in affecting primary tumors as well as in preventing their metastasis and recurrence.[43] Li et al. synthesized a nanodevice for the delivery of thrombin to cancer cells. They developed a rectangular DNA origami sheet with thrombin-DNA conjugates which were then loaded into DNA origami nanotubes. The developed nanorobot served as an excellent platform for the delivery of the therapeutic thrombin in tumor blood vessels to efficiently block the tumor blood supply and inhibit the growth of the tumor cells. The nanorobot also prevented metastasis along with the inhibition of primary cells and thus exhibiting a promising therapeutic potential.[44]

4. Proteins

Nanoparticles based on protein biopolymers, also known as protein nanoparticles, are widely used in pharmaceuticals due to their stability from enzymatic degradation and efficiency.[45,46] Proteins are a type of natural molecule present in all living organisms, including hemoglobin, digestive enzyme, tubulin, and insulin. Protein nanoparticles can be synthesized using proteins such as lipoprotein, legumin, and ferritin proteins. These nanoparticles have good properties like high surface area, low melting point, low percolation threshold, etc. These particles can form covalent bonds with drugs and ligands.[47–49]

Proteins are polymers of amino acids formed by a peptide linkage. This is a condensation reaction along with the removal of water molecules. The human body has around 100,000 different types of protein.[50] Introducing the drug with or in nanomaterials is called drug loading. Nanoparticles used in drug delivery will have drug load capacity. High drug loading capacity should minimize the number of administrations or doses.[51] Dispersibility is required for easy and efficient delivery of the medicine. Drug loading can be done by many methods. Drug loading and trapping efficiency include nanoparticle drug solubility, the dispersion medium, size and composition of nanomaterials, molecular weight (MW) and solubility of drugs,the interaction of drug nanomaterials, and presence of surface functional groups(amine, ester, etc.) either on drugs.[52,53]

Many nanostructures in a drug delivery system are formed by self-organization, a force balancing process in which well-defined structures form spontaneously from building blocks.[54] The main driving forces in the self-organization process are non-covalent interactions such as London forces, electrostatic interactions, π- stacking interactions, steric forces, and solution and lubrication.[54] Compared to covalent bonds, non-covalent interactions are weak, involving more scattered variations of electromagnetic interactions between atoms or within molecules.[55]

It is important to be aware of the composition of proteins and peptides to cope with various problems while developing a drug delivery system. Proteins are relatively larger molecules with complex structures. The peptides chains between peptides and proteins are rarely linear and adapt a variety of distinctive fold 3-D patterns and conformation.[56]

Nanoparticles are slowly biodegradable, which can lead to systemic toxicity and reduce the ability to adjust doses.[57] Nanoparticles can be used in targeted drug distribution to improve the availability of poorly soluble drugs,[58,59] the targeting drugs at a specific site, and drug bioavailability.

The use of nanoformulations of anti-cancer drugs to obtain individualized drugs is an attractive approach, especially in cancer treatment. Different types of cancer have emerged in recent years. Cancer has affected various tissues and areas such as the breast, bones, and blood.[60] Unfortunately, efforts such as chemotherapy, radiotherapy, immunotherapy, and invasive surgeries can only prolong a patient's survival and rarely cure cancer completely, mainly due to lack of proper diagnosis, poor understanding of various causes and mechanisms of cancer, and lack of effective treatment.[61]

Apolipoprotein E has been proposed to mediate the transport of drugs into the bloodstream - a blood-brain barrier[62] loaded into human blood albumin nanoparticles and bound to apolipoprotein E. The effectiveness of this drug delivery system is of course based on the recognition of lipoprotein receptors.Kopelman and his team have created Encapsulated probe on their surface, to carry a variety of unique active ingredients and perform multiple functions[63] by biologically local embedding (pebble).

The main challenges to nanoformulations are ease of production of a sterile form, managing critical micelle concentration, and stability of nanoparticles which plays a major role in the ultimate function of the nanoparticles. These include proteins of the immune system and complement systems, including immunoglobulin. Enormous efforts are being made to control the interaction of drug formulations of various proteins to improve the effectiveness of nanomedicine.[64–67]

Several nano biosensors are being considered to find various kinds of protein antibodies, and enzymes are used to diagnose the disease.[68–70] For example, antibodies are used to find infections, and the immobilized enzyme glucose oxidase is used in the production of glucose nano biosensors.

5. Co-delivery of multiple molecular systems

Combining various medications or treatment techniques to improve therapeutic impact and lessen the side effects of cancer therapy is a popular research topic. Different co-delivery systems must be designed and built following the various structures and properties of cargoes and vectors. The delivery system is essential for achieving combination therapy. The diverse structures of medications and vectors give a plethora of design directions for the co-delivery systems. The interaction of cargoes and carriers, as well as the synergistic impact of loaded

reagents, should all be taken into account. Following the property and structures of goods and carriers, appropriate modalities or cooperative techniques must be chosen. Physical procedures for encapsulating reagents into vectors are usually simple to use. However, the stability of such delivery methods must be thoroughly investigated. Chemical techniques produce relatively stable delivery systems.[71]

5.1. Design Considerations

While a combination of several therapeutic modalities provides the best therapeutic impact on cancer, increasing demands are being placed on the rational design of delivery systems. Vectors that deliver only one type of drug are no longer sufficient for cancer treatment. Vectors conveying two or more types of reagents referred to known as co-delivery vectors have been developed to realize combination therapy akin to clinical therapies (Figure 7). For cancer treatment, nanoparticles or micelles deliver doxorubicin (DOX) and PTX at the same time. Nanoparticles like Poly (D, L-lactide-co-glycolide acid) containing vincristine sulfate and verapamil hydrochloride improve tumor cell susceptibility to chemotherapeutic treatments. Gene treatment, phototherapy, and immunotherapy can all be integrated by loading diverse reagents such as nucleic acid, photosensitive chemicals, and quantum dots into co-delivery vectors. Multiple chemotherapeutic medications are given in a sequential order in the clinic to improve chemotherapy efficacy and delay MDR. The surface of the vectors is conjugated with targeting molecules to increase the targeting of vectors to the lesion location; the vectors can then be loaded with imaging agents to accomplish diagnosis and therapy integration.

Figure 7. Co-delivery systems and collaborative treatment strategies. Reproduced from ref.[72], Copyright (2020), with permission from Elsevier.

Loading methods of drug molecules can be categorized into chemical and physical processes. The most prevalent physical interactions are hydrophobic interaction, electrostatic interaction, and simple encapsulation. Whereas in chemical reactions, to enable the regulated release of intact drug molecules, stimuli-responsive bonds, as well as structures, should be created between the backbone and the drug molecule.[72]

In the tumor microenvironment, the bonds or structures that may be activated must be carefully planned. This procedure allows for the controlled discharge of cargoes. More drug delivery systems with potential performance and therapeutic benefits will be available, along with the advent of breakthrough technologies including the use of molecular machines in drug delivery systems. A synergistic effect can be achieved by administering different medications in the correct order. Combining anti-metabolites and taxanesin the right order, for example, can turn an antagonistic impact into a synergistic effect.[72]

5.2. Chemically Modified DNA Nanostructures

DNA nanostructures with metal nanoparticles and quantum dots (QDs) were extensively investigated for years. Nanoparticles of various types and sizes were co-assembled on a DNA nanostructure via interactions like Au–S bond, DNA base-pairing, and biotin-avidin interaction.[15] The four types of modification procedures based on the varied positions of functional groups or molecules in the modification of DNA tetrahedrons include vertex, capsule, mosaic, and cantilever. The binding of functional chemical modifications to the apex of a DNA tetrahedron such as altering the three vertices of a DNA tetrahedron to a thiol group is known as a vertex-type modification. The "AuS" link and the DNA tetrahedron have a stable three-dimensional pyramid structure, allowing the DNA tetrahedral nanomaterial to be stably attached to the gold surface.[73]

Simple self-assembly, stable mechanical properties, better biocompatibility, and stability in the presence of ribozymes are only a few of the benefits of functionalized DNA tetrahedral nanomaterials. They have promising biosensing, separation analysis, bioimaging, and drug delivery applications.[71] Because of DNA's programmability, numerous outstanding multi-dimensional DNA nanostructures, such as two-dimensional or three-dimensional nanostructures, have been created for drug delivery. Stable transport, targeted drug delivery, drug localization, and nanocarrier release are all goals of stimuli-responsive drug delivery systems with DNA nanostructure, which significantly increase therapeutic efficacy while reducing harmful side effects on normal tissues.[74]

Drug delivery is a prospective application of these chemically modified DNA nanostructures due to their enhanced stability and related functional moieties as a result of chemical changes. The systematic distribution of chemotherapeutic and nucleic acid drugs has been documented using several rationally designed drug delivery methods based on chemically modified DNA nanostructures. They discovered that the size and form of chemically modified DNA nanostructures must be

carefully designed to achieve efficient cellular absorption and macrophage evasion.[13]

Simple mixing of four single-strand DNAs (ssDNA) yielded tetrahedral DNA (TDNs). TDNs utilized for encapsulating anticancer medicine doxorubicin (DOX) are highly ordered constructed nanostructures with uniform size, facile production, and great drug loading efficiency. Redox responsive polyethyleneimine (PSP) and TDNs@DOX nanocomplexes of diameter 240 nm were created as a result of the electrostatic interaction. The molar ratio of nitrogen to phosphorus (N/P) was a key factor in determining cell size, zeta potential, and the in-and-out pathway. The PSP/ TDNs @DOX NCs (N/P = 30) gradually disassembled due to disulfide breaking in response to intracellular high concentrations (> 10 mM) of glutathione (GSH) at the tumor location. NCs that had been deconstructed and had a smaller size (less than 50 nm) penetrated deeply into tumor tissues.[75]

Chemically modified DNA nanostructures usually have better enzymatic stability, which means they last longer. Finally, chemically modified DNA nanocarriers can easily add diverse targeting and programmable release features using covalent conjugation. Chemically altered DNA origami with numerous changeable sites, in particular, has a lot of promise for precisely co-loading and efficiently co-delivering diverse therapeutic medicines via a protective shell that boosts nuclease resistance. In 2019, Guo et al. grafted a phosphorothioate-modified DNA backbone with benzyl bromide-modified paclitaxel (PTX) to accurately load chemotherapy drugs. The amphiphilic PTX-DNA hybrid structure can form spherical nucleic acid-like micellar nanoparticles with ease. Hybridization and sequence design can easily incorporate a targeted aptamer and a functional antisense strand into this nanoparticle. In vivo, this multifunctional nanoparticle shows active-targeting delivery and effective tumor growth suppression.

Firstly, chemically modified DNA can help with structure assembly while also avoiding the creation of numerous DNA strands. Secondly, additional covalent bonds could be employed to enhance the material's functionality. Third, DNA nanostructures that are chemically modified are frequently more enzymatically stable, extending therapeutic efficacy. Finally, chemically modified DNA nanocarriers can easily add diverse targeting and programmable release features using covalent conjugation. This type of rationally designed drug delivery method is important for reducing systemic toxicity and improving medication pharmacodynamics.[13]

The toolbox of supramolecular chemistry provided the researchers with well-defined orthogonal interactions that can steer DNA assembly into novel architectures, leading to hybrids with greater functionality and complexity.[71]

CpG motifs are unmethylated cytosine-phosphate-guanosine sequences that have been shown to trigger macrophage immunological responses by activating Toll-like receptor 9(TLR9). CpG motifs were added to a tetrahedron as well as an origami scaffold after this discovery, and they were demonstrated to boost immune activation. Through orthogonal interactions, synthetic insertions have also been employed to guide the supramolecular assembly of DNA. Wang et al. and Haner et al., for example, have used pi-pi stacking interactions to create foldable DNA polymers. Similarly, the conjugation of dendrons to DNA produced novel building blocks that were found to improve duplex stability, scaffold lipid vesicle production, and facilitate long-range assembly. Yan, Liu, and colleagues also used amphiphilic ligands to decorate DNA origami structures, resulting in cuboid and dumbbell-shaped vesicles. Hermann, Gianneschi, and colleagues demonstrated that DNA-block copolymer assemblies produce micelles that are controlled by molecular recognition between DNA bases.[71]

5.3. Dual Fluorescence Imaging-Guided Programmed Delivery System

To alter the tumor microenvironment for successful chemo-immunotherapy, a dual fluorescence imaging-guided delivery system containing CpG nanoparticles and DOX was developed. In comparison to the direct administration of doxorubicin from the hydrogel, CpG self-crosslinking nanoparticles from a hydrogel ensured a long-lasting immune-stimulating impact. Immune cells from the tumor microenvironment, such as cytotoxic CD8+ T lymphocytes, myeloid-derived suppressor cells, and M2-like tumor-associated macrophages, were studied further to uncover the likely mechanism of chemo-immunotherapy. Because of the co-stimulation of doxorubicin and CpG nanoparticles, the tumor microenvironment was positively steered toward tumor-suppressive circumstances, resulting in a higher immune response for effective chemo-immunotherapy. Furthermore, the fluorescence of doxorubicin and genipin crosslinking CpG nanoparticles was used to detect dual fluorescence imaging-guided programmable administration.[76]

Dual fluorescence imaging guiding chemo-immunotherapy would provide accurate data for optimizing the scheduled co-delivery of chemotherapeutic medicines and immunostimulants (Figure 8). Fluorescence imaging in vivo provides a particular advantage over other imaging modalities such as MRI, US, CT, and PET for monitoring many medications at the same time because of its multispectral fluorescence splitting.[76]

5.4. Co-delivery of Multiple Molecules

To achieve a synergistic impact of the combination of cytotoxicity and immune stimulation, a well-designed combination approach should address the correct medication selection, dose schedule, and method of administration. According to cross-sectional examinations of biomaterials and pharmaceutics for cancer immunotherapy, the intervention of biomaterials provides good feasibility for scheduled co-delivery of chemotherapeutic medications and immunostimulants. When compared to DOX-CpG NP and the GEL-DOX treatment the antitumor effects of GEL-CpG NP-DOX chemo-immunotherapy were highlighted in two ways: (1) The introduction of GEL increased the bioavailability of medicines and adjuvants by extending the action period of DOX-CpG NP and achieving a sustained planned release of DOX and CpG NP. (2) DOX-induced tumor cell destruction released a large number of tumor-associated antigens or "warning" signals, which boosted

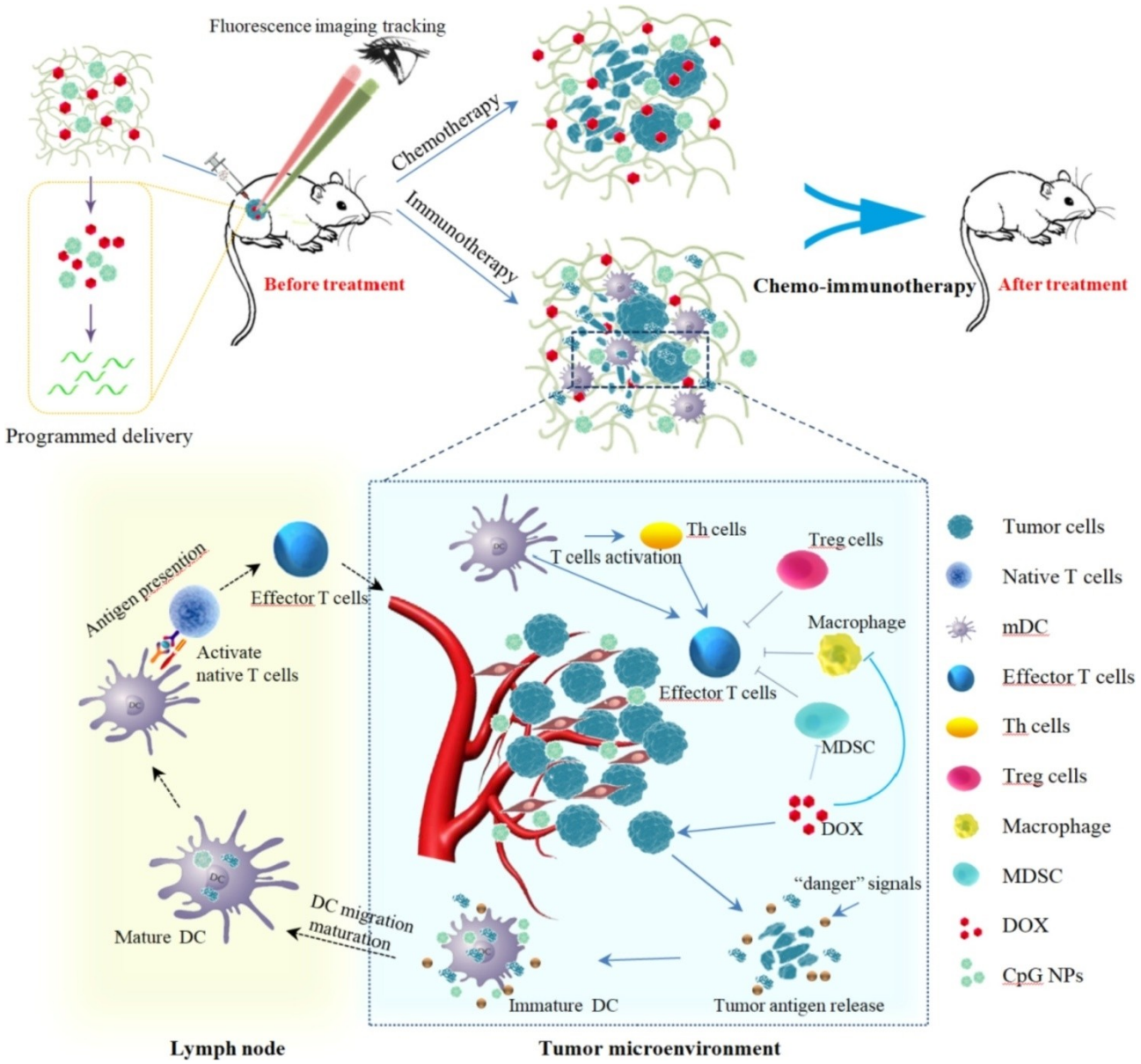

Figure 8. Scheme of chemo-immunotherapy based on imaging-guided programmed delivery of DOX and CpG nanoparticles from a hydrogel. Reproduced from ref.[76], Copyright (2020), with permission from Elsevier.

the immune system's reaction, and CpG NP boosted the immune response even more. Using these strategies, chemo-immunotherapy with GEL-CpG NP-DOX produced the best tumor suppression. For DCs to achieve antigen presentation, a combination of DOX and CpG NP was required.[76]

Only the perspective of curative impact makes it difficult to prove whether the interaction induced by medication combinations is synergistic. To assess the combined effect of medications, numerous scientific evaluation approaches have been applied. Cell viability data were analyzed using CompuSyn software to examine the potential synergy between DHA, DOX, and TS. The findings revealed an antagonistic relationship between DHA and DOX with CI values ranging from 0.5 to 0.9, and fractions affected (Fa) ranging from 1.32 to 1.55. Similarly, the synergy study demonstrated that the combination of DOX, DHA, and TS exhibited synergistic effects as well, demonstrating that the inclusion of DHA did not lessen the formulation's synergistic effect. It's worth noting that various studies have found DHA to help reduce the adverse effects of chemotherapy, particularly anthracycline-induced cardiotoxicity as well as improve cancer prognosis. Because of the protective role of DHA and TS against the negative effects of DOX, NLC-DHA-DOX-TS could result in higher anticancer efficacy and decreased toxicity as a result of the drug's synergistic impact. In vitro release tests revealed that the NLC releases DOX in a regulated manner, with enhanced drug release in an acidic environment. DOX, DHA, and TS mixed in NLC show synergistic actions against 4T1 tumor cells, according to in vitro cell

research. Furthermore, when compared to the free drug, DOX encapsulation in this NLC improved cellular absorption. The in vivo analysis revealed that this formulation not only slowed the growth of 4T1 breast cancer tumors, but it also lowered mouse mortality, prevented lung metastasis, and reduced DOX-induced heart and liver damage. Finally, our findings support the hypothesis that NLC loadingDHA, DOX, and TS can be considered a potential formulation for breast cancer treatment.[77]

Also used tubular and planar DNA origami nanostructures to bind two types of enzyme molecules, glucose oxidase (GOx) and horseradish peroxidase (HRP). They devised a catalytic cascade in which the result of the first reaction acts as the substrate for the second. Because the distance between two enzymes determines the efficacy of the cascade, it is feasible to fine-tune it by connecting the enzymes to the nanostructure at various positions. Their cascade reactivity was greatly improved when they were encased in a DNA nanotube, a phenomenon resulting from the constrained nano space, which mimicked the congested intracellular milieu. This approach paves the path for a novel method of delivering a high-efficiency, well-organized nanoreactor to a cell.[17]

Liposomes, which may carry both hydrophilic and lipophilic medicines, are important carriers in the DDS sector (Figure 9).Lipophilic medications are partitioned to the lipid bilayer, while hydrophilic drugs are transported in the inner space of liposomes. Liposomes may hypothetically deliver various medicines of varying lipophilicity simultaneously. Liposomes are also incredibly adaptable because cationic liposomes form complexes (lipoplexes) with nucleic acids and plasmid DNA.[78]

Multiple drug co-delivery systems are a promising cancer-fighting strategy. Co-delivery systems are predicted to provide synergistic effects and fewer negative effects.Carrier designs that respond to the environment can manage cargo release. Multiple medications with diverse modes of action are used in combination therapy to improve efficacy and reduce the severity of side effects. In combination therapy, a suitable co-delivery strategy is critical, although the dosage schedule (regimen) for co-delivery systems is easier than that for combination therapy. Co-delivery systems can simplify clinical processes and increase patient QOL, despite their more difficult preparation than mono-delivery systems. Recent advancements have been made in stimuli-responsive smart DDS, notably those that combine physical stimuli with nanoparticles delivered to precise locations in target tissues. Using DDS in combination with therapy would boost efficacy and safety even more. The simultaneous administration of various medicines to the same (cancer) cells is likely to have both additive and synergistic effects. There are a variety of medication combinations available, each with a particular mechanism of action, albeit the combinations may not always match therapeutic regimens. Although co-delivery of medications with distinct qualities is difficult, several researchers have succeeded in developing such systems.[78]

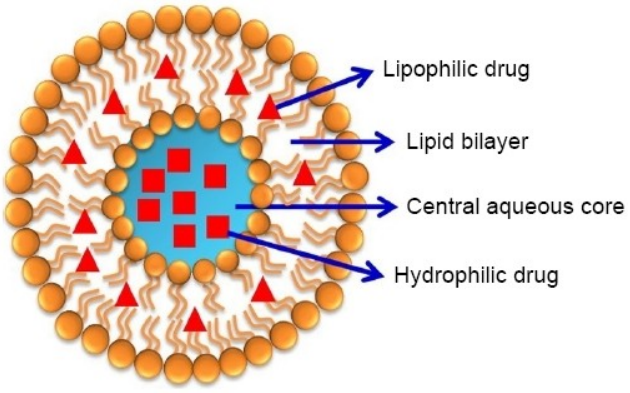

Figure 9. Diagrammatic representation of liposome particles. Reproduced from ref.[52], Copyright (2017), with permission from Dove Medical Press.

6. Challenges and Perspective

DNA nanostructures have proven to be a promising platform for addressing many challenges in biomedicine (Figure 10).[79] They also provide several advantages over conventional polymeric materials or other nano-objects. The ease of synthesis, the programmability, control over size and shape, and the ability to deliver multiple agents have attracted the

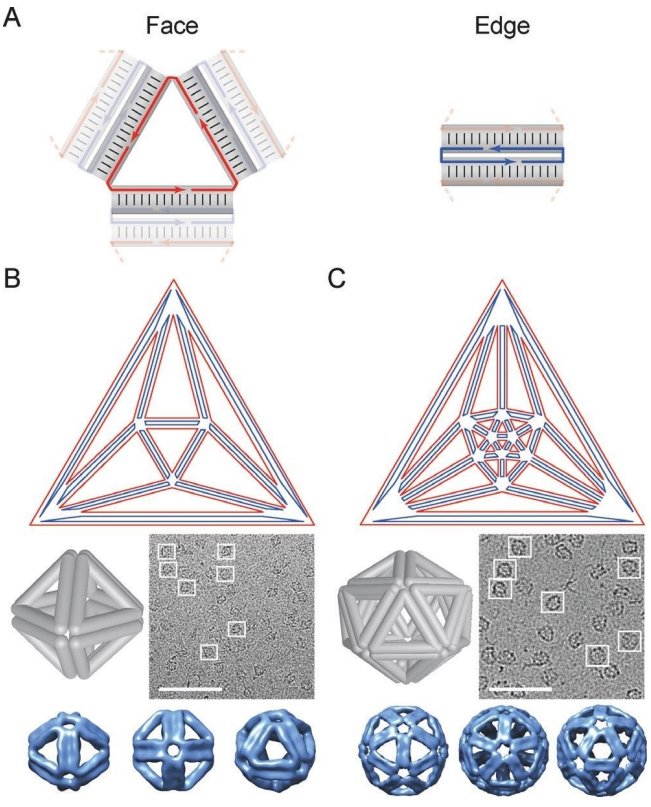

Figure 10. DNA polyhedral. (A) schematic diagrams of the representative face and edge in polyhedron (B) DNA octahedron and (C) DNA icosahedrons. Cryo-EM images on the middle row (scale 100 nm): different views of 3D maps of DNA polyhedra constructed from cryo-EM images on the bottom row. Reproduced from ref.[4], Copyright (2019), with permission from Wiley-VCH.

scientific community to study their potential in cellular delivery.[80] Due to homogeneity and compatibility between the cargo and the carrier, the use of DNA nanostructure for the delivery of nucleic acids is the most natural way to think of.[17] But several challenges have to be addressed in the application of DNA nanostructures in clinical practice.

One prerequisite for DNA nanostructures for biomedical applications is their stability in the living system. DNA nanostructures are susceptible to the attack of nucleases and their stability in long-term incubation sometimes remains a challenge. The digestion of DNA nanostructures depends on their mechanical properties and structure. But the structures can be stabilized by many strategies of coating and crosslinking. These improvements may result in nanostructures that are irresponsive to external stimuli which can be corrected by the application of stimuli-responsive coating/cross-linking. Sometimes to maintain their structural integrity inorganic particles like Mg^{2+} are used. However, the concentration of the particle required may be high for the cellular environment.[81,82]

Another challenge remains in the difficulty to synthesize long DNA sequences. Large-scale synthesis by DNA origami requires a large number of staple DNA strands which increases the cost of production. Even though the modifications on the surface increase their targeting and other properties, these modifications may result in some difficulty while loading and release of the drugs.[81] In response to the stimuli, some structural changes occur to the DNA nanostructures enabling the release of drugs. These intracellular structural changes of DNA nanostructures need to be monitored in real-time. Structural switch of these assemblies has only been demonstrated in hypothetical situations ignoring the complex biological environment in living systems.[82] Further, the heterogeneity of DNA nanostructures may cause some side effects. Precise dosing is challenging, particularly for intercalating medicines whose loading and release profile rely on a variety of factors.[83]

The understanding of the mechanism by which the DNA is internalized by the cells also remains a challenge. Due to the relative scarcity of DNA nanostructures that have been evaluated for endocytosis, the endocytosis mechanism of DNA nanostructures is not well understood. Over recent years, there have been some advancements in understanding the process and pathways of endocytosis but the chemical endocytic pathway inhibitors conventionally used are nonspecific. Furthermore, it has been shown that some pathways can crosstalk and inhibition of one pathway may result in the upregulation of another in compensation.[79,83]

In general, DNA nanotechnology has a lot of room for advancement and the field will quickly be expanded for various biomedical applications. AdvancedDNA nanostructures that combine various triggering systems will enable more precise diagnostics and addressable drug delivery. Targeting a complicated diseased environment with different stimuli-responsive DNA nanocarriers can reduce the effectiveness of the off-target effect. It's a promising strategy for intelligent regulation of therapeutic behaviors by developing sequential stimuli-responsive procedures for directing the various processes of intracellular transport.[82] The characterization of the fate of DNA nanostructures in biological conditions will help us to select the most promising strategies and choices. The rate of degradation, the bio-distribution, the structure-activity relationships, and the interaction of the DNA nanostructures with living cells are needed to be extensively studied for the better and more efficient application of DNA nanostructures.[80] A comprehensive understanding of the interaction between living organisms and DNA nanostructures is very essential to understanding the endocytosis mechanism and the pharmacodynamics and pharmacokinetics of DNA nanostructures. Big data analytics and machine learning can help with this exploration. DNA nanostructures has the potential to act as immunosuppressant to regulate the levels of cytokines whoch otherwise may cause permanent organ damage or even death.[83] Finally, a standardized reproducible protocol mat results in the fast advancement in the field of nanomedicine. The use of different protocols, models, and inconsistencies in the results slow down the development of nanoparticles for nanomedicine. Therefore, a standardized and reproducible testing approach is urgently needed to connect the impact of DNA nanostructures with their biological outcomes.[80]

Despite some limitations present, DNA nanotechnology is a rapidly developing research field. As a result, DNA nanotechnology-based therapeutics have the potential to be apromising platform for clinical therapeutics. A multidisciplinary approach is required to accelerate the development of DNA nanotechnology.[80,83]

7. Conclusion

DNA-based drug delivery systems have gained prominence due to their high cellular uptake efficiency, programmability, and biocompatibility. In this review, we enumerate various DNA nanostructures used for therapeutic molecule delivery. The functionalization of DNA carriers, which makes it possible to create integrated drug delivery platforms, broadens the scope of nanostructure applications. We also discussed the new architectures created by supramolecular chemistry, which opens up new possibilities for future design strategies that are beyond the scope of this review.

Acknowledgements

We acknowledge the support from the Department of Chemistry, Sacred Heart College (Autonomous), Thevara and Kairali Research Award (2020) from Kerala State Higher education Council, Government of Kerala (FJ). We thank Ms. Anjaly Rajeev for her assistance in graphic design.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Co-delivery · DNA nanostructures · DOX · mRNA · siRNA

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Submitted: August 2, 2022
Accepted: August 25, 2022