Nucleic acid–based aggregates and their biomedical applications

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Abstract
The more than three decades of research in nucleic acid nanotechnology has led to the thrilling progress in rationally designed structures and artificial molecular devices with programmable functions and various applications. Nucleic acid–based aggregates feature precise molecular recognition and sequence programmability, versatility, as well as marked biocompatibility, providing promising candidates for biomedical applications. In this minireview, we summarize the recent, successful efforts to construct and employ nucleic acid–based aggregates for biomedical applications, including drug delivery, bioimaging, biosensing, cell analysis, and combined cancer therapy. We also discuss the remaining challenges and opportunities in the field.

KEYWORDS
biomedical applications, fabrication, nucleic acid–based aggregates, self-assembly

1 | INTRODUCTION
Nucleic acids (DNA and RNA), as essential biomacromolecules, possess many distinct and attractive properties, such as biocompatibility, biodegradability, precise molecular recognition (Watson–Crick base pairing and noncanonical interactions), and sequence programmability.[1] Owing to these characteristics, nucleic acids have been broadly exploited to build a variety of tile-based nanostructures including two-dimensional arrays,[2] polyhedral,[3] and 3D crystals[4] as well as origami-based nanostructures.[5] Small bricks and large origami structures have been used as building blocks to fabricate complex nucleic acid structures of various geometries, such as teddy bears[6] and rings,[7] through multivalent hydrogen bonding and π–π stacking. Much progress has been reported in utilizing nucleic acid–based materials for multiple biomedical applications, including drug delivery, biosensing, and bioimaging.[8] The advances of tile-based and origami-based nanostructures have already been reviewed in great detail.[1a,8a,b,9]

Aggregation is an important process, commonly taking place in biological systems or material world, which also serves as a widely used “bottom-up” technique for the fabrication of nanomaterials. Nucleic acids can work as building blocks or linkers to construct and regulate different molecular aggregates. Apart from nucleic acid structures with specific geometries, nucleic acid–based aggregates refer to micro-/nanostructures without precisely controlled size and shape, such as dendrimers,[10] hydrogels,[11] microsponges,[12] nanoflowers (NFs),[13] nanoclews,[14] and nanocapsules.[15] According to their compositions and the role of nucleic acids, nucleic acid–based aggregates can be classified into two main types: (1) nucleic acid–formed aggregates and (2) nucleic acid–assisted aggregates. Nucleic acid–formed aggregates are structures made up of nucleic acids, which serve as both building blocks and cross-linkers. Nucleic acid–assisted aggregates stand for composite structures consisting of other nanomaterials as the major building blocks and cross-linkers. Nucleic acid–assisted aggregates serve as widely used “bottom-up” technique for the fabrication of nanomaterials.

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biomedical applications of these nucleic acid–based aggregates, including drug delivery, bioimaging, and biosensing.

2 NUCLEIC ACID–FORMED AGGREGATES

Based on their aggregating mechanisms, nucleic acid–formed aggregates can be subdivided to two parts: aggregates that produced by (1) nucleic acid tiles-based ligation or hybridization and (2) rolling circle amplification (RCA)/rolling circle transcription (RCT)-derived assembly.[16]

2.1 Nucleic acid tiles-based aggregates

2.1.1 Construction of nucleic acid tiles-based aggregates

Most nucleic acid hydrogels are fabricated with cross-linked nucleic acid tiles via either enzyme-catalyzed ligation or programmable complementary hybridization.[16,17] One of the main obstacles in the production of such hydrogels is the connection of the branched nucleic acid tiles. To address this issue, Luo and co-workers developed a DNA hydrogel constructed entirely with branched DNA tiles, which can be cross-linked through enzyme-catalyzed ligation.[11a] The authors designed and hybridized DNA strands to form branched DNA tiles with X, Y, and T shape, which served as building blocks (Figure 1A). These branched DNA tiles, possessing complementary sticky ends with palindromic sequences, were combined and connected using T4 DNA ligase to form a larger-scale and three-dimensional DNA hydrogel. In addition, efficient encapsulation and continuous release of camptothecin (CPT) and porcine insulin were achieved. This study introduced these biodegradable DNA hydrogels formed conveniently by ligation as a new sort of aggregate materials that can be employed for diverse applications, especially sustained drug delivery.

Hybridization is another main approach to cross-link nucleic acid tiles. In 2009, Liu’s group designed a pH-responsive DNA hydrogel by incorporating i-motif DNA as both cross-linkers and responsive switches.[11b] These DNA hydrogel aggregates were assembled by Y-shaped branched DNA tiles through the construction of intermolecular i-motif complex triggered by mildly acidic environment (Figure 1B). The Y-shaped tiles contained pH-sensitive, cytosine-rich i-motif domains for the association of neighboring i-motif. These intramolecular i-motif structures would remain as random coils at pH values over 6 owing to their electrostatic repulsion, while shift to a more stable state as the triple hydrogen bond forms between the unprotonated and protonated cytosines at lower pH values (pH < 6). To visualize the gelling transformation, red-colored AuNPs were added to the solution before initiating the process. The AuNPs were trapped within or released from the gel, subjected to the state transition induced by pH changes. Due to the slight acidity of tumor microenvironment, the pH-responsive DNA aggregates have been successfully applied in effective drug delivery and controlled release for cancer therapy.[18]

Based on simple cross-linker hybridizations, Dirks and Pierce[19] developed the hybridization chain reaction (HCR), which provides an alternative approach to construct tiles-based aggregates. Upon introduction of a triggering strand, the hairpins undergo a cascade of strand displacement reactions, resulting in long polymeric nanowires or dendritic nanostructures.[20] In 2016, Fan and coworkers reported a clamped HCR system for DNA hydrogels production. Unlike conventional linear and nonlinear HCR, this system contained a special hairpin strand carrying palindromic segments, which formed hairpin-dimers after annealing (Figure 1C). Importantly, the hairpin-dimer with two branches could form three-arm or four-arm junctions for subsequent deviating chain reactions, and eventually led to a clamped DNA hydrogel.[21] This HCR-based strategy was also adopted by Zhang’s group to construct DNA-PEG (poly (ethylene glycol)) bottlebrushes composed of PEGylated hairpins. The introduced PEG side chains effectively improved the enzymatic stability and pharmacokinetics of shielded DNA backbones.[22] In 2019, Willner and coworkers reported a DNA network with fascinating stiffness-switchable features based on HCR. The DNA network was composed of four toehold-modified constituents, which act, respectively, as bidentate units for chain-elongating and tetradentate structure for cross-linking. Determined by the balance between bidentate and tetradentate units in the presence of varied counter effectors, the hydrogels were switched across different stiffness states. The dynamic networks exhibited self-healing properties using diverse previously reported ancillary triggers including heat, light, and pH, which could be utilized for tissue engineering and construction of bioadhesion materials.[23]

Additionally, some reagents and metal ions that interact with nucleotide bases have been employed to control the hybridization of tiles-based aggregates. In 2013, Luo and colleagues introduced psoralen, a natural reagent that intercalates and cross-links DNA, to Y shape DNA tiles. The resultant thermostable nanostructures were utilized as modular primers for polymerase chain reaction (PCR) to produce extended DNA networks.[24] In 2014, a noble metal ion, Ag+, was exploited by Willner’s group to realize switchable formation and dissociation of DNA hydrogels (Figure 1D). The intermolecular cytosine-Ag+-cytosine bridges resulted in the cross-linking of DNA tiles and therefore the construction of the hydrogels, while the removal of the Ag+ from the bridges using the cysteamine ligand would dissociate the hydrogels.[25] More recently, the chemical cross-linking reaction between guanines was employed by Luo and coworkers to transform biomass DNA into biodegradable materials. They displayed a new class of DNA aggregates cross-linked by the nitrogen–carbon bonds formed between poly (ethylene glycol) diacrylate (PEGDA) and guanines within DNA strands. The DNA aggregates could be converted to diverse materials including gels, membranes, and plastics and have been utilized for drug delivery, unusual adhesion, multifunctional composites, patterning, and everyday plastic objects.[26]

2.1.2 Biomedical applications of nucleic acid tiles-based aggregates

The aforementioned nucleic acid tiles-based aggregates formed by cross-linking of DNA tiles via ligation or hybridization provide an effective and practical platform for
biomedical applications, such as cell-free protein production, cell analysis, and targeted drug delivery.

As per the central dogma of molecular biology, DNA is transcribed into messenger RNA (mRNA), which then serves as a template for protein translation. Luo and coworkers constructed enzyme-catalyzed nucleic acid aggregates and incorporated genes into the gel scaffolds to create a hydrogel for protein production (P-gel). This P-gel was fabricated by connecting X shape DNA tiles and linear templates via T4 ligase (Figure 2A). Interestingly, the P-gel aggregates displayed higher efficiency and better yield than previously optimized solution-phase systems (SPS). To understand the underlying mechanism of P-gel’s superior performance, further investigation was performed and indicated that the cross-linked hydrogel protected gene from DNase digestion. The compressed domain enhanced transcription efficiency through increased local gene concentration, as gene proximity induced a faster enzyme turnover rate compared with most other cell-free SPS, in which the linear templates were dispersed in solution. Notably, the DNA hydrogel system presented potential as a universal and efficient platform for protein production and provoked the development of an RNA-producing hydrogel aggregates, termed as I-gel, for RNA interference (RNAi).
Based on the outstanding features including specific molecule recognition, stimuli-responsive properties and triggered release, nucleic acid–formed aggregates have been exploited for single cell analysis. Single cell analysis represents the accurate interpretation of biological systems with cellular heterogeneity at the single-cell level, rather than examine the average information derived from large populations of cells.[30] To realize permeable cover and controlled release of single living cells, Liu’s group developed an enzyme-triggered DNA network made of Y shape tiles and linkers as a shroud for microwells.[31] This DNA network could be specifically cut by enzymes due to restriction sites introduced into the linker strands (Figure 2B). Compared with conventionally used glass or polydimethylsiloxane (PDMS) membranes, the DNA network was penetrable for nutrients and waste, which maintained the cells enveloped in the microwells viable, providing a platform to culture, monitor and manipulate individual cells.

Nucleic acid aptamers are single-stranded DNA or RNA with a length ranging from 10 to 100 nucleotides (nt), which are produced from a process termed “systematic evolution of ligands by exponential enrichment” (SELEX).[32] Owing to their specific recognition of biological compounds, aptamers have been incorporated with tiles-based and origami-based nanostructures for applications such as targeted drug delivery and bioanalysis.[33] Because of their facile modular design and assembly, tile-based aggregates were also integrated with aptamers for bioimaging and cancer therapy. For instance, Tan and colleagues designed a stimuli-responsive DNA nanohydrogel as an effective targeted gene delivery vector. Functional elements, including cell-targeted aptamers, antisense oligonucleotides capable of inhibiting cell proliferation and disulfide linkages for controllable release inside cells, were incorporated into the DNA aggregates (Figure 2C). As expected, treatment with this DNA nanohydrogel led to a marked hindrance of cell proliferation and
migration.\cite{34} Besides, aptamers were employed as stimuli-responsive oligonucleotide shells of microcapsules for drug delivery and controlled release. In a work of Willner’s group, an anti-ATP (adenosine triphosphate) aptamer was utilized as bridging units of DNA layers outside the microparticle template (CaCO$_3$).\cite{35} While encountering ATP trigger, energetically stabilized ATP-aptamer complexes were formed, resulting in the dissociation of the microcapsules and targeted release of encapsulated cargoes.

As an important member of therapeutic nucleic acids, oligodeoxynucleotides (ODNs) containing unmethylated cytosine-phosphate-guanosine (CpG) sequences were also employed to functionalize tile-based aggregates. CpG ODNs with immunostimulatory effects, which can activate antigen-presenting cells (APCs) through toll-like-receptor 9 (TLR9), have been used as adjuvants for immunotherapy.\cite{36} Based on these facts, Ding and coworkers constructed DNA dendritic aggregates as delivery platform to carry immunostimulatory CpG motifs.\cite{37} TAT peptides and Loop CpG ODNs were connected with the DNA dendrimers to improve the cell membrane permeability and enhance the stability of the CpG ODNs (Figure 2D). In vitro experiments demonstrated that cell internalization and cytokines production were strengthened, while no obvious cytotoxicity was observed, suggesting that the DNA dendrimers can act as designable and reliable delivery vehicles for the immune modulators.

The CRISPR/Cas9 platform, which is a crucial component of the RNA-directed defense system in prokaryotes, has been exploited as a facile gene-editing tool to specifically modify or correct the genome for gene therapy.\cite{38} This platform merely involves endonuclease Cas9 and an engineered, single-guide RNA (sgRNA).\cite{39} However, appropriate carriers to deliver CRISPR-Cas9 platform still hinder further therapeutic applications. In 2019, Ding’s group developed branched DNA tiles-based aggregates to transport sgRNA, Cas9, and antisense ODNs together (Figure 2E). Further morphology studies revealed that, unlike conventional hydrogels with amorphous internal structures, this meta-hydrogel was built hierarchically, which might contribute to its unique solid- and liquid-like properties.

RCA/RCT reactions involving two circular templates complementary to each other provide an alternative method for the fabrication of conventional RCA/RCT-derived aggregates. In 2014, Lee’s group produced an RNA membrane through two sequential processes, complementary RCT, and evaporation-induced self-assembly (Figure 3C). The structural and functional properties of the membrane were rationally controlled by adjusting RNA base pairing.\cite{41} In a follow-up study, they adopted partially complementary RCT to produce bubbled RNA aggregates.\cite{45} The bubbles allowed the RNA aggregates to contain multiple dicer-cleavage sites to release the functional siRNAs after being internalized to cells.

\subsection*{2.2.2 Biomedical applications of RCA/RCT-derived nucleic acid aggregates}

RCA/RCT-derived aggregates, including DNA/RNA microsponges, DNA NFs, nanoclews, and nanocapsules, possess intriguing properties, including manufacturing convenience, high yields, tunable sizes, versatility, and remarkable cargo-loading efficiency.\cite{16, 17b} Varieties of functional nucleic acids, fluorophores and anticancer chemodrugs, are able to be integrated into RCA/RCT aggregates within the enzymatic processes or through complementary hybridization.\cite{13a}

Therapeutic nucleic acids, including siRNA, short hairpin RNA (shRNA), and antisense oligonucleotides (ASO),\cite{46} can efficiently be constructed into nucleic acid aggregates. In 2012, Hammond and coworkers synthesized RCT-derived RNA microsponge aggregates as delivery vehicles of shRNA
FIGURE 3 Fabrication of RCA/RCT-derived nucleic acid aggregates. (A) Stepwise approach for DNA hydrogel production through RCA and MCA reaction. Reproduced with permission.© Copyright 2012, Nature Publishing Group. (B) D-, N-, and A-shaped hydrogels with solid-like property were successfully formed and tested by removing and replacing water. Reproduced with permission.© Copyright 2012, Nature Publishing Group. (C) RNA membrane produced through complementary RCT. Reproduced with permission.© Copyright 2014, Nature Publishing Group

In their design, hairpin RNA strands produced by RCT were self-assembled into nanoscale pleated sheets that could be further condensed to generate sponge-like microspheres, termed RNA microsponges.[12a] These microsponges were composed entirely of cleavable RNA strands. After being internalized by cells, the microsponge-like aggregates were cleaved by the ribonuclease, Dicer, into siRNA with the length about 21 nts. The siRNAs were subsequently unwound into single strands and interacted with several cellular enzymes to form the RNA-induced silencing complex for gene silencing. Additionally, positively charged polyethylenimine (PEI) was coated on the RNA aggregates to enhance cellular uptake. This study displayed a much more accessible route for RNAi therapy, which not only protected the siRNA from degradation during delivery but also was capable of achieving high siRNA loading efficiency.

A similar strategy mentioned above has also been applied to the effective delivery of antisense ODN. In 2014, Hammond’s group described an RCA-derived DNA microsponge made up of composite nucleic acid/magnesium pyrophosphate.[12b] A size favorable for effective cellular and systemic ODN delivery was obtained by encapsulating the microsponge with cationic polymer, poly-L-lysine (PLL), which displaced the magnesium pyrophosphate crystals from the aggregates (Figure 4B). Additional layers of negatively charged short ssDNA and positively charged endosomal escape agent, PEI, coated the outside of the PLL/ODN core through electrostatic interaction. The multifunctional DNA microsponge demonstrated greater nuclease stability and prolonged in vivo half-life, serving as a less toxic delivery system for efficient antisense therapy.

Taking use of built-in multifunctional moieties, RCA/RCT-derived aggregates were utilized to multiple biomedical applications. In 2013, Tan and coworkers developed non-canonical monodisperse DNA NFs with densely packed DNA by RCA.[13a] During the RCA process, fluorophores were incorporated into the NFs by attaching fluorophores to either primers or dNTPs (Cy5-dUTP) via chemical modification (Figure 4C). The incorporation of fluorophores was confirmed through fluorescence microscopy, which assured the feasibility of using DNA NFs for bioimaging.[13b] After further combination with cancer cell-targeting aptamers and an
Anticancer drug, the resultant DNA NFs were applied for targeted drug delivery to cancer cells.[13c,47]

RCA-derived aggregates can also be adopted as a vehicle for simultaneous delivery of Cas9/sgRNA platform for gene editing. In 2015, Gu’s group synthesized yarn-like DNA nanoclewes formed by ultralong DNA strands, whose template sequences were designed to be partially complementary to the 5′ end of sgRNA, resulting in a reversible interaction.[14b] Cas9 and the sgRNA were combined together and then loaded on the nanoclews. Fluorescence microscopy images and flow-cytometry analysis revealed that the CRISPR-Cas9 delivered by the nanoclews yielded the highest genetic disruption efficiency (Figure 4D). Similar results were obtained after further evaluation of the in vivo EGFP interference potency in a U2OS.EGFP tumor-bearing mouse model, which indicated the DNA nanoclew aggregates had thrilling potential as a new delivery platform for targeted genome editing with CRISPR-Cas9.

Over the past decades, encouraging progress have been made in cancer immunotherapy, that is, treatments that modulate the immune system to treat cancer.[48] Cancer immunotherapy usually adopts the following strategies: checkpoint inhibitors (anti-CTLA4, anti-PD-1, and anti-PD-L1),[49] adoptive transfer of combined antigen receptor...
(CAR) T cells,[50] and tumor-infiltrating T lymphocytes (TILs).[51] These approaches usually require the combination of multiple strategies to realize synergistic therapeutic effects. In their inspiring study, Chen and colleagues presented a peeling DNA-RNA nanocapsule as a nanovaccine that synergistically delivered CpG ODNs and shRNA adjuvants together with tumor-specific neoantigens for cancer immunotherapy.[15] CpG and shRNA were produced via simultaneous RCA and RCT techniques (Figure 4E). The products were then intertwined into DNA-RNA nanoflower-like aggregates, which were further shrunk by PEG-grafted cationic polypeptides and loaded with neoantigen to form the nanovaccine. Significant inhibition of the proliferation of neoantigen-specific colorectal tumor and sustained memory T-cell production were achieved, confirming the potential of this versatile tumor immunotherapeutic nanovaccine.

In addition to efficient and targeted delivery of various therapeutics, RCA-derived aggregates have also been employed for stem cell fishing. Bone marrow mesenchymal stem cells (BMSCs), a widely investigated type of stem cells, exhibit important biological functions, such as differentiation into various cell types, homing to sites of injured tissues, and immune regulation.[52] Yang’s group developed a double RCA network formed not only by physical entanglement but also by hybridization between DNA chains.[53] Two ultralong DNA chains were first generated separately as precursors from two partially complementary circular DNA templates, then mixed and intertwined with each other to generate 3D DNA network aggregates (Figure 4F). The molecular diffusion and phase inversion in the mixture were visualized by the color change of independently stained DNA chains during the DNA network formation process. Aptamer Apt19S was incorporated into this DNA network. This aptamer possesses high affinity with ALPL overexpressed on the cell membrane and, most importantly, size-related electronic and optical properties depending on particle distance, which can be controlled by specific DNA linkers. While introduced to other nucleosides, the aggregates were stable and showed purple hue. When adenosine was detected, the aptamer-based DNA switch can bind two adenosine molecules, inducing disassembly of the DNA-AuNPs aggregates and resulting in a red color of individual DNA-AuNPs.

A similar approach was also used for colorimetric detection of Hg2+ in aqueous media.[57] In 2007, Mirkin and coworkers developed two classes of AuNPs modified with varied DNA sequences, which were entirely complementary except for a thymidine–thymidine mismatch (Figure 5B). Upon encountering Hg2+, which selectively coordinates with the T-T mismatch bases, significantly more stable DNA-AuNPs aggregates formed, increasing the melting temperature (T_m) of the resulting structures. Subsequently, quantitative detection of Hg2+ with high selectivity and sensitivity was efficiently realized by the color change of the solution at given temperatures based on the dynamic changes of DNA-AuNPs aggregates.

Aside from detection purpose, AuNPs was decorated with functional and stimuli-responsive DNA strands to construct dynamic aggregates for combinatorial anti-cancer therapy.[58] In 2018, Kim and colleagues designed a nanomachine in which G-quadruplexes and i-motif DNA structures served, respectively, as loading sites for photosensitizers and pH-responsive domains for AuNPs dynamic aggregation (Figure 5C). Furthermore, the authors loaded the chemotherapeutic drug, doxorubicin (DOX), into the duplex DNA by intercalation. Once the duplex DNA unwound, the drug was efficiently released. Consequently, combined chemotherapy, photothermal, and photodynamic therapy was realized with the DNA-regulated AuNP aggregates. In these cases, the function of nucleic acid and AuNP was further expanded.

3 | NUCLEIC ACID–ASSISTED AGGREGATES

Aside from nucleic acid–formed aggregates consisting of nucleic acids serving as both building blocks and cross-linkers, nucleic acids-assisted aggregates represent another aggregate class. Nucleic acids in this kind of aggregates have been utilized to regulate the aggregation states of other nano-materials including AuNP, IONPs, and GO for various applications, including detection, imaging, photodynamic, and photothermal therapy. Based on their composition, nucleic acid–assisted aggregates can be divided into (1) AuNPs–nucleic acid aggregates, (2) IONPs–nucleic acid aggregates, and (3) GO–nucleic acid aggregates. In these cases, nucleic acids are usually modified to interact with other nanomaterials and serve as cross-linkers and functional units.

3.1 | AuNPs-nucleic acid aggregates

As the most mature and stable metallic nanomaterials, AuNPs have been well developed for numerous applications including drug delivery, catalysis, imaging, and detection owing to their outstanding properties.[54] AuNPs demonstrate various fascinating features such as tunable size and shape, great biocompatibility, facile surface modification and, most importantly, size-related electronic and optical properties arising from resonant oscillation of free electrons in the presence of light, known as localized surface plasmon resonance (LSPR).[55]

Taking advantage of LSPR and easy modification through the strong interaction between thiol and gold, AuNPs have been functionalized with thiolated nucleic acids and applied in a variety of aggregation-based optical biosensing platforms. In 2006, Lu and coworkers designed adenosine-sensitive aggregates[56] consisting of two DNA-functionalized AuNPs (DNA-AuNPs) and a DNA aptamer linker (Figure 5A). DNA-AuNPs have a high extinction coefficient and exceptional optical properties depending on particle distance, which can be controlled by specific DNA linkers. While introduced to other nucleosides, the aggregates were stable and showed purple hue. When adenosine was detected, the aptamer-based DNA switch can bind two adenosine molecules, inducing disassembly of the DNA-AuNPs aggregates and resulting in a red color of individual DNA-AuNPs.

IONPs consisting of organic or inorganic shells and Fe_3O_4 core have been demonstrated to be promising magnetic resonance imaging (MRI) contrast agents.[59] Based on their relaxation effects, MRI contrast agents can be used either as T1 contrast agents producing bright signals in the delivered regions or as T2 contrast agents leading to dark signals. Conventional IONPs with strong magnetization are usually
FIGURE 5 Examples of construction and applications involving nucleic acid–assisted aggregates. (A) Aptamer-functionalized gold nanoparticle aggregates for the colorimetric detection of adenosine. Reproduced with permission.[56] Copyright 2006, Nature Publishing Group. (B) DNA-modified gold nanoparticles for colorimetric detection of Hg$^{2+}$ in aqueous media. Reproduced with permission.[57] Copyright 2007, Wiley-VCH. (C) DNA–AuNP aggregates equipped with i-motifs and G-quadruplexes for triple combinatorial cancer therapy. Reproduced with permission.[58] Copyright 2017, Wiley-VCH. (D) pH-responsive iron oxide nanocluster aggregates for MRI-based diagnosis of small hepatocellular carcinoma. Reproduced with permission.[61] Copyright 2018, American Chemical Society. (E) DNA-directed aggregation of graphene oxide for the ultrasensitive oligonucleotide detection. Reproduced with permission.[63] Copyright 2011, American Chemical Society.

employed as T2 contrast agents for the detection of tumor lesions in the liver, while ultrasmall IONPs with a diameter less than 4 nm have been applied as T1 MRI contrast for detection of small tumor regions.[60] Therefore, by controlling the aggregation states of small IONPs, T2/T1 transformation can realize stimuli-responsive MRI, providing an inverse contrast enhancement to improve the early-stage diagnosis of small hepatocellular carcinomas (HCCs).

Based on this principle, Ling’s group fabricated an intelligent MRI contrast agent, termed as responsive iron oxide nanocluster assembly (RIA). The aggregates of the DNA-modified ultrasmall IONPs were constructed by cross-linking IONPs with pH-responsive i-motifs.[61] In vivo MRI verified the inverse contrast enhancement between the dark normal liver tissue in the presence of IONP aggregates and bright HCC due to the disassembly of ultrasmall IONPs. Compared with their irresponsible counterparts, RIAs brightened the tumor 2 h after injection because of the dissociation of the IONP aggregates in the acidic tumor microenvironment (Figure 5D). By introducing pH-responsive i-motif linkers to IONPs, the system showed the thrilling potential of stimuli-responsive nucleic acid in the development of responsive MRI contrast agents with inverse contrast enhancement properties.

3.3 | GO–nucleic acid aggregates

GO, a water-soluble derivative of graphene, holds various advantages such as straightforward synthesis, good water dispersibility, and facile surface modification. It has been demonstrated that single-stranded DNA and peptides can bind to GO via $\pi-\pi$ stacking and hydrogen bonding, while DNA duplexes and aptamers do not exhibit powerful connection to GO because of unfavorable thermodynamics.[62]

In 2011, Li and coworkers reported a method of controllable assembly of GO nanosheet aggregates using DNA hybridization.[63] Two DNA–GO complexes were formed separately by adding single-stranded DNA to GO solutions, respectively. When cDNA linkers that hybridize with the two
complexes were added to the mixture, the GO assembled into layered nanosheets with tunable interspaces (Figure 5E). In particular, the size of GO aggregates was determined by cDNA concentration and sequence. Therefore, qualitative and quantitative cDNA detection was realized by this homogeneous biosensing platform based on GO aggregates.

To sum up, nucleic acid has acted as cross-linker and functional unit to modify other nanomaterials and control their aggregation states for biomedical applications including detection, imaging, and cancer therapy. The prosperous development of these nucleic acid–assisted aggregates may inspire new designs for programmable and multifunctional cancer therapeutic agents.

4 CONCLUSIONS AND FUTURE PERSPECTIVES

Nucleic acid–based aggregates have been successfully utilized to fabricate rationally designed nanostructures with wide applications. Here, we summarized the recent progress in the construction and employment of nucleic acid–based aggregates as novel and effective theranostic platforms. On the one hand, nucleic acid–based aggregates share common advantages with nucleic acid nanomaterials, such as inherent biocompatibility, biodegradability, programmability, site addressability, and precise molecular recognition.[1a,8a] On the other hand, nucleic acid–based aggregates possess several unique characteristics, including convenient manufacturability via enzymatic reactions, the capability of massive production, high drug-loading efficiency, and enhanced stability.[8b] Empowered by their innate versatility, nucleic acid–based aggregates enable the engineering of multiple functional nucleic acids into a single nanostructure, which is especially adept at co-delivering multiple nucleic acid therapeutics for combined cancer therapy.[64] Multiple functional moieties, including aptamers, siRNA, shRNA, antisense oligonucleotides, and immunostimulatory CpG motifs, can be efficiently attached or integrated into nucleic acid–based aggregates.[65]

Though various nucleic acid–based aggregates have been successfully developed and evaluated, several crucial challenges remain to be addressed before further applications. Precise size control within the nanometer scale is the main obstacle hindering the clinical translation of nucleic acid–based aggregates.[66] This critically affects the cellular uptake of aggregates and the delivery of functional molecules to the cytoplasm.[13a] Although cation polymers, such as PEI and PLL, have been employed to shrink and condense the aggregates generated via RCA or RCT, the side effects and toxicity limit the wide application of these aggregates.[12] Moreover, although RCA/RCT-derived aggregates have resistance against degradation and excellent drug loading efficiency, poor release efficiency of cargoes causes another problem to therapeutic performance. Additionally, detailed in vivo characteristics of nucleic acid–based aggregates, such as circulating half-life, pharmacokinetics, and clearance mechanisms also require to be elucidated.

Inspired by the aggregation of nucleic acid–formed and assisted structures, we are now able to control the aggregation states under biological conditions, regulating physiological and pathological functions. For instance, by adjusting the activity of blood coagulation protease, thrombin, researchers have succeeded in regulating the activation and aggregation of fibrinogen to affect coagulation and anticoagulation.[67] In our previous work, we constructed a tubular DNA nanorobot functionalized with thrombin within its inner cavity for targeted tumor vessel occlusion and efficient cancer therapy. In vivo experiments demonstrated that intravenously injected DNA nanorobots delivered thrombin specifically to tumor-associated blood vessels and induced intravascular thrombosis, resulting in tumor necrosis and inhibition of tumor growth.[33c] Manipulating the aggregation states of biomacromolecules may provide a novel approach to understand and treat thrombosis-related and neurodegenerative diseases.[67,68] We envision that multifunctional and biologically amenable nucleic acid–based aggregates will serve as powerful tools in future biomedical studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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