DNA: From Carrier of Genetic Information to Polymeric Materials

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Abstract

Research has shown that the DNA molecule can not only store genetic information but also serve as a polymeric biomolecule for the fabrication of functional materials. The unique precise molecular recognition capability and sequence programmability, combined with its good biocompatibility and biodegradability, impart the DNA molecule considerable potential for use in the construction of multifunctional materials. Depending on the composition, DNA-based materials have been generally categorized into pure DNA materials that are entirely composed of DNA and hybrid DNA materials that are composed of DNA and other functional compositions. Recently, we have developed a series of DNA-based materials that can be applied in diagnosis and therapy, and this review summarizes the relative work. Although challenges still exist regarding the real applications of DNA-based materials such as the high cost of DNA, the difficulty in scale-up, and the low resistance to nuclease, we believe that these drawbacks will be overcome with the development of technology, and new opportunities will emerge in the field of diagnosis and treatment.

Keywords DNA materials · DNA nanotechnology · Biomedicine

Introduction

In the past few decades, remarkable progress has been made in the research pertaining to the DNA biomolecule. The DNA strand is made up of four types of nucleotides connected by phosphodiester bonds, and each nucleotide is composed of a N-containing nucleobase (cytosine [C], guanine [G], adenine [A], or thymine [T]), a deoxyribose, and a phosphate group. The DNA double helix structure was demonstrated by Watson and Crick in 1953 [1]. As a genetic molecule, DNA can transmit genetic information from generation to generation owing to its capacity to self-replicate. In addition, genetic information can be translated to protein, which has been demonstrated in cell-free systems. In this context, we had earlier concentrated nucleic acids in clay hydrogel to mimic the confinement function of cells in a cell-free system (Fig. 1A) [2]. We observed enhanced

expression of the fluorescent protein in the DNA/clay hydrogel compared to that in the free DNA system, which confirmed the occurrence of improved transcription and translation reaction. The work implicated the importance of localized concentration and protection of biomolecules in early life evolution. We also fabricated a clay microgel as an artificial cell and incorporated plasmid DNA into it to achieve efficient protein production (Fig. 1B) [3]. In this system, we used the microfluidic droplet technology to prepare plasmid-binding clay microgel beads in a modular manner. We observed that the eGFP protein was expressed only in the droplets encapsulating the plasmid/clay hydrogel. Moreover, magnetic nanoparticles were also incorporated in the plasmid/clay hydrogel matrix to construct a repeated protein production system through magnetic separation, thus providing an efficient and repeated cell-free protein production system.

In addition to being a carrier of genetic information, DNA has also been used as a biopolymer to construct multifunctional materials owing to its unique properties such as precise molecular recognition capability, molecular programmability, enzyme operability, and good biocompatibility and biodegradability [4–8]. It is well known that two complementary DNA strands can hybridize into a double helix structure through Watson–Crick base pairing, as follows: adenine [A] pairs with thymine [T] and

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Fig. 1 Genetic expression in a cell-free system. **A** (a) Scheme of transcription and translation reactions that occurred in the clay hydrogel environment, (b) the preparation of clay hydrogel, and (c) luminescence images of RLuc protein expressed in the clay hydrogel. Reproduced with permission [2]. Copyright 2013, Nature Publishing

Group. **B** (a) The fabrication of clay microgel-based cell-like structure via microfluidic droplet technology, (b) fluorescence images of the artificial cell with eGFP expression. Reproduced with permission [3]. Copyright 2018, American Chemical Society

guanine [G] pairs with cytosine [C]. The specific pairing of DNA bases enables it with programmable sequences, which is the guidance for the fabrication of DNA materials [9–11]. Furthermore, there is a rich toolbox of enzymes for DNA manipulation and modification, such as nuclease, polymerases, and ligases, which can facilitate the assembly and functionalization of DNA-based materials. Moreover, DNA amplification technology is a powerful method that can be used to construct DNA-based materials. For example, polymerase chain reaction (PCR) is one of the common techniques used for the rapid amplification of a specific DNA molecule in vitro, which can produce more than a million target DNA molecules in a few hours. In addition, rolling circle amplification (RCA), an isothermal

nucleic acid amplification method, can utilize a circular DNA template to synthesize a long and repetitive DNA product in a few minutes using DNA polymerase. The abovementioned unique advantages make DNA a promising material in DNA nanotechnology.

In the following content, we shall focus on our recent work on the construction of DNA-based materials and their applications in diagnostics and therapy. Depending on the components of the substrate, the DNA materials we have prepared are divided into two categories, pure DNA and hybrid DNA. Pure DNA materials are entirely made up of DNA, whereas hybrid DNA materials are the combinations of DNA and other functional materials for special applications.

DNA Materials

Pure DNA Materials

Pure DNA materials are primarily fabricated based on self-assembly and DNA amplification technology. For example, we synthesized a thermostable branched DNA primer through DNA self-assembly and then fabricated a bulk DNA hydrogel from the cross-linking branched DNA produced by PCR (Fig. 2) [12]. In detail, we designed three single-strand DNA (ssDNA) molecules with partially complementary sequences and then assembled them into a branched DNA structure (Fig. 2A). After treating with psoralen, the branched DNA (Y-DNA) became thermostable and could be used as a modular primer in PCR for extension in multiple directions (Fig. 2B). When forward Y-DNA primers and reverse Y-DNA primers were introduced in the same PCR system, a bulk DNA hydrogel was fabricated for Y-DNA-formed three-dimensional networks (Fig. 2C). This work provided a PCR-based approach for the fabrication of various DNA materials.

In another work, we constructed a pure DNA hydrogel composed of elongated single-strand DNA through DNA chain elongation using the RCA method as shown in Fig. 3A [13]. In the RCA process, Φ 29 DNA polymerase used circular DNA as a template for the RCA reaction to synthesize a long ssDNA molecule, followed by multiprimed chain amplification (MCA). After running RCA and MCA, the DNA chains got tangled up to form a DNA hydrogel. Unlike other hydrogels, the scanning electron

microscope (SEM) images demonstrated that the DNA hydrogel had a hierarchical internal structure like a bird nest (Fig. 3B). The shear-storage modulus (G') was constantly higher than the shear-loss modulus (G''), which confirmed that it was a gel (Fig. 3C). Unexpectedly, the gel exhibited unusual properties, i.e., when taking out the gel from water, it became liquid-like and accumulated at the bottom of the glass bottle; however, when put back into water, it displayed solid-like properties and returned to its original shape (Fig. 3D). This work developed a new strategy to fabricate DNA meta-hydrogel using polymerase enzyme, and the gel had potential applications in manufacturing electric switches and flexible circuits.

Hybrid DNA Materials

Hybrid DNA materials are receiving more attraction because of their improved properties. Due to its properties of molecular recognition capability and sequence programmability, the DNA molecule can serve as a scaffold for the construction of metal nanoparticles [14–16]. We synthesized a branched DNA/AgNC (super-AgNC) with fluorescence that exhibited improved antibacterial performance (Fig. 4A) [17]. The process included two steps: First, we designed three strands of C-rich sequence ssDNA as templates to prepare ssDNA/AgNC, and second, the ssDNA/AgNC was used as a building block to construct branched DNA/AgNC via self-assembly. The fluorescent property of the super-AgNC was tunable (Fig. 4B). In addition, the super-AgNC demonstrated enhanced antibacterial performance compared with Ag^+

Fig. 2 Branched DNA synthesized by PCR for the fabrication of DNA hydrogel. **A** Schematic illustration of the synthesis of thermostable branched DNA structure, **B** elongated products of branched DNA, and **C** formation of DNA hydrogel by PCR of branched primers. Reproduced with permission [12]. Copyright 2013, Wiley-VCH

Fig. 3 DNA hydrogel. **A** Schematic diagram of the formation of the DNA hydrogel, **B** SEM image of the DNA hydrogel, **C** mechanical property of the DNA hydrogel, and **D** liquid- and solid-like properties

of the DNA hydrogel. Reproduced with permission [13]. Copyright 2012, Nature Publishing Group

(Fig. 4C). The (Y-X)-super-NC even delayed the bacterial growth by 8 h at the appropriate concentration.

Then, we created a fluorescent hybrid DNA hydrogel by incorporating AgNC DNA into the RCA-elongated DNA strands (Fig. 5A) [18]. We first designed a circular DNA as a template for the RCA reaction to produce elongated DNA chains, which generated a hydrogel network through physical entanglement. The AgNCs were formed at the DNA scaffolds in the RCA gel, resulting in a hybrid DNA hydrogel. Due to the optical property of AgNCs, the resultant hybrid gel exhibited fluorescence under UV light [Fig. 5A(b)]. The SEM image demonstrated that the hybrid gel possessed a typical fiber-like structure [Fig. 5A(c)], which was different from the bird nest-like structure that we had reported previously. The good biocompatibility and fluorescence properties of the hybrid RCA gel endow it with great potential for use in biosensing, bioimaging, and tissue engineering. Other

nanomaterials such as magnetic nanoparticles have also been incorporated into the DNA hydrogel to endow them with novel properties. In our recent work, we developed a type of magnetic DNA nanogel by fabricating an RCA-DNA nanogel layer on the surface of magnetic nanoparticles (MNPs) (Fig. 5B) [19]. The TEM images demonstrated an obvious DNA layer coating on the MNPs [Fig. 5B(b)], and the surface of the hybrid nanogel was rough as observed by SEM [Fig. 5B(c)]. Our magnetic DNA nanogel exhibited good cellular uptake as well as low cytotoxicity, and therefore, it could be used as a vector for controlled drug delivery. This work provided a universal method for fabricating a hybrid DNA nanogel that is responsive to stimuli such as magnetism, electricity, and light.

The nucleobase and the phosphate groups in the DNA molecule can coordinate with metal ions to form DNA-metal hybrid materials with diverse applications in biosensing,

Fig. 4 DNA as a template for the synthesis of AgNCs. **A** Schematic illustration of different DNA/AgNCs: ssDNA/AgNC, Y-super-NC, X-super-NC, and (Y-X)-super-NC, **B** optical properties of (Y-X)-

super-NC, and **C** *Escherichia coli* growth curves in the presence of different DNA/AgNCs. Reproduced with permission [17]. Copyright 2018, Wiley-VCH

Fig. 5 Hybrid DNA hydrogel. **A** (a) Schematic diagram of the synthesis of the hybrid DNA-AgNC hydrogel, (b) fluorescence photograph of the hybrid DNA-AgNC hydrogel under UV light exposure, and (c) SEM image of the hybrid DNA-AgNC hydrogel. Reproduced

with permission [18]. Copyright 2018, Wiley-VCH. **B** (a) Schematic representation of DNA-MNP nanogel, (b) TEM image of DNA-MNP nanogel, and (c) SEM image of DNA-MNP nanogel. Reproduced with permission [19]. Copyright 2018, American Chemical Society

Fig. 6 Self-assembly of lanthanide ions and thymidine. **A** Scheme for the synthesis of luminescent ultralong microfibers through SSA of lanthanide and thymidine in water, and **B** fluorescent microscopy images of ultralong microfibers. Reproduced with permission [23]. Copyright 2018, Wiley-VCH

bioimaging, drug delivery, and enzyme catalysis [20–22]. We have recently created luminescent ultralong microfibers through supramolecular self-assembly (SSA) of lanthanide ions and nucleoside thymidine [23]. Thymidine was used as a ligand to chelate lanthanide ions Eu(III)/Tb(III) to form luminescent complexes in water, and then, the complexes were able to further assemble into ultralong luminescent microfibers (Fig. 6A). The microfibers exhibited bright characteristic luminescence of Eu(III)/Tb(III) on exposure to ultraviolet light because thymidine can effectively protect and sensitize Eu(III)/Tb(III) in water (Fig. 6B). These microfibers were rarely a centimeter long and were obtained by supramolecular self-assembly and could have significant potential in optical applications.

DNA-Based Biosensors

The superiority of DNA materials endows them with significant potential in biomedicine, especially in biosensing. Recently, we have made considerable efforts to promote the practical applications of DNA-based sensors in the field of diagnostics and therapy.

Molecular diagnostics is an important technology that can be applied in the diagnosis and monitoring of diseases. Various desirable characteristics of diagnostics assays require to be satisfied, including high specificity, high sensitivity, less time consumption, reproducibility, and multiplex detection capability [24]. The unique properties of DNA, for example, sequence programmability, enable ssDNA to hybridize with specific nucleic acids; especially, the DNA aptamer can recognize and combine with protein. These properties impart

Fig. 7 Nucleic acid detection with the branched DNA probe. **A** Schematic illustration for the synthesis of CCLB-DNA, **B** preparation and regeneration process of the biosensing interface, **C** the frequency responses of the biosensing system to T-DNA, **D** the linear relation-

ships between the frequency shifts and $\lg[\text{T-DNA}]$ (fM), and **E** frequency shifts of the biosensing system in four cycles. Reproduced with permission [26]. Copyright 2018, Elsevier B.V

DNA-based materials a significant potential in the diagnosis of diseases [25]. We have recently developed a recyclable nucleic acid detection system using branched DNA as a probe based on special recognition of DNA (Fig. 7) [26]. We first constructed a chemically cross-linked branched DNA (CCLB-DNA) with sticky ends for specially capturing target DNA (T-DNA). The CCLB-DNA was immobilized on the gold chip of a quartz crystal monitor. The hybridization

of CCLB-DNA and T-DNA would generate an electrical frequency shift, and further hybridization of M-Fe₃O₄ and T-DNA could generate a signal amplification. The biosensing system exhibited linear relationships between the frequency shifts and $\lg[\text{T-DNA}]$ (fM). It was noteworthy that the chemically branched DNA probe could ensure structural integrity under denaturation conditions; therefore, it enabled to recycle in practical applications.

In another work, we further employed the branched DNA-based SERS platform to achieve early multiplex diagnosis of

Fig. 8 DNA-based sensor for the detection of microRNAs and protein. **A** Schematic of modified AgNF for the assay of both AFP and miRNA, **B** AFP detection was analyzed by Raman spectra, and **C**

miRNA detection was analyzed by Raman spectra. Reproduced with permission [27]. Copyright 2018, American Chemical Society

liver cancer (Fig. 8) [27]. The silver nanoparticle film was modified with reporter domains, and both Y-DNA/ssDNA and anti-AFP were bound to reporter domains for capturing

microRNA and AFP, respectively. This was the first time that a branched DNA was used for microRNA detection. Remarkably, compared with normal ssDNA, the branched

Fig. 9 DNA-based protein detection system. **A** Schematic diagram of the DNA-based protein detection system and **B** using the universal adapter for multiplexed protein detection. Reproduced with permission [28]. Copyright 2013, American Chemical Society

DNA enabled two orders of higher sensitivity for the detection of microRNA, and the limit of detection was as low as 10^{-17} M. Based on the ultrasensitive and multiplex performance, this branched DNA-based biosensor demonstrated great potential in the detection of nucleic acids.

Protein detection has a great significance in disease diagnosis and differentiation in clinical medicine. Currently, western blotting, enzyme-linked immunosorbent assay, and immunohistochemistry are the common methods for protein detection, in which primary antibodies and labeled secondary antibodies are used. However, the limited availability of primary and secondary antibody pairs seriously hinders the advancement of protein detection. To address this challenge, we developed a universal DNA-based protein detection system (Fig. 9) [28]. A protein-DNA hybrid molecule was constructed and used as a universal adapter (UA) for protein detection. The UA was composed of a binding protein for antibodies and a DNA-binding moiety. The binding protein could bind most types of IgG, and the DNA-binding moiety could hybridize with a specific DNA reporter. Therefore, using UA, it was possible to establish a library of pre-labeled primary IgG antibodies for any application of protein detection without the requirement of secondary antibodies.

Gastric cancer is an extremely aggressive tumor. Traditional diagnostic techniques for gastric cancer primarily include endoscopy, biopsy, and blood tests. These techniques are generally complex and time-consuming. To address these issues, we have recently constructed a biosensing system based on luminescent DNA/Ag nanoclusters (DNA/AgNCs) for the detection of D-amino acids (DAAs) (Fig. 10) [29]. The detection process was dominated by two reactions, D-amino acid oxidases

(DAAO) stereo specifically catalyzed DAA oxidation and Fe^{2+} -catalyzed Fenton reaction (Fig. 10A). The coupling of DAA oxidation and Fenton reaction demonstrated tunable fluorescence quenching of DNA/AgNCs to realize rapid and noninvasive detection of DAA with chiral recognition (Fig. 10B, C). For a sample test, the fluorescence of DNA/AgNCs was quenched only in the presence of target DAAs in the case of testing simulated gastric juice and saliva samples, which suggested a high selectivity toward DAA in the presence of interferences. This work provided a strategy for the diagnosis of markers associated with gastric cancer, allowing for point-of-care testing.

Conclusion and Outlook

In this review, we have summarized our recent research on the construction of DNA-based materials and their biomedical applications, including diagnosis and therapy. We envision that the unique molecular recognition capability and the sequence programmability of the DNA molecule will help us to construct more desirable functional materials for specific applications. Although challenges such as high cost of DNA, difficulty in scale-up, and low resistance to nuclease still exist, the development of science and technology will certainly remove these impediments in the future.

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Fig. 10 DNA-based detection of D-amino acids. **A** Schematic diagram of the DAA-based diagnosis system, **B** validation of the fluorescence quenching of the probe, **C** specificity of DAA detection, **D** diagnosis of simulated gastric juice and saliva, and **E** effects of interferents in gastric juice and saliva on detection. Reproduced with permission [29]. Copyright 2017, The Royal Society of Chemistry

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