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Responsive DNA-based hydrogels and their applications

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Abstract

The term hydrogel describes a type of soft and wet material formed by crosslinked hydrophilic polymers. The distinct feature of hydrogels is their ability to absorb a large amount of water and swell. The properties of a hydrogel are usually determined by the type of polymer and crosslinker, the degree of crosslinking, and the water content. However, a group of hydrogels, called “smart hydrogels”, changes properties in response to environmental changes or external stimuli. Recently, DNA or DNA-inspired responsive hydrogels have attracted considerable attention in construction of smart hydrogels because of the intrinsic advantages of DNA. As a biological polymer, DNA is hydrophilic, biocompatible, and highly programmable by Watson-Crick base pairing. DNA can

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form a hydrogel by itself under certain conditions, and it can also be incorporated into synthetic polymers to form DNA-hybrid hydrogels. Functional DNAs, such as aptamers and DNAzymes, provide additional molecular recognition capabilities and versatility. In this review, we discuss DNA-based hydrogels in terms of their stimulus response, as well as their applications.

Keywords

DNA; hydrogel; responsive

Introduction

A hydrogel is a type of crosslinked hydrophilic polymer that can absorb a large amount of water and undergo a volume change. Before crosslinking, the hydrophilic polymers are dissolved in water without a defined shape. Covalent or noncovalent crosslinks lead to formation of gels which can maintain solid-like 3-dimensional structures, while the high solvent content within the gel gives rise to fluid-like properties. Typically, the type and degree of crosslink, as well as the type of polymer, define the properties of a hydrogel.

Hydrogels can be classified as stimuli-responsive or non-responsive gels. Non- responsive hydrogels passively absorb water and swell. Stimuli-responsive or intelligent hydrogels sense surrounding environmental changes and respond accordingly. Hydrogels controlled by pH [1], temperature [2], electric field [3], and ionic strength [4] have attracted attention because of their potential in biosensing and biomedical applications. However, hydrogels that only rely on polymer properties have limited responsive capabilities; therefore, a key research goal is modification to achieve versatile stimulus response. The incorporation of molecular recognition motifs, including antibodies [5], polypeptides [6], polysaccharides [7], oligonucleotides [8], and enzymes [9], into hydrogels has expanded the available stimuli.

Of particular interest are DNA-based hydrogels based on the unique features of nucleic acids. Nucleic acids are polymers of nucleotides that are well known as genetic materials. The discovery of Watson-Crick base pairing and the development of fully automated chemical synthesis in the late 1970s [10] have expanded the role of DNA from purely biological molecules to attractive chemical materials. The purine and pyrimidine base pairs establish the fidelity of double helix structure, which makes oligonucleotides programmable and predictable polymers in materials science [11]. The first work utilizing DNA as a building block in nanotechnology was carried out by Seeman [12] who assembled DNA into four-arm Holliday junctions and lattices, proving that DNA can be made into artificial structures. Since then, DNA has been studied in many fields beyond biology. Different nanostructures, nanomachines and smart materials have been made with DNA for various applications.

In addition to Watson-Crick pairing to form double-stranded DNA from ssDNA, oligonucleotides can serve as functional units known as aptamers and DNAzymes. Aptamers are single-stranded oligonucleotide (or peptide) molecules chosen from a random library by an iterative screening method called Systematic Evolution of Ligands by EXponential enrichment (SELEX) [13, 14]. They are often regarded as chemical antibodies because they

can specifically recognize a wide range of targets that vary from small molecules to cancer cells [13–23]. The dissociation constants (Kd) of aptamers are in the low nanomolar to high picomolar range, comparable to the K s of antibodies [24]. Since their discovery in the 1990s, the development of aptamers as specific, high-affinity probes with diagnostic and therapeutic applications has been widely studied [25]. Aptamers can be chemically conjugated with various signal-generating components for acoustic [26–28], electrochemical [29–32], and optical [33–35] sensing. In addition, by taking advantage of adaptive recognition, aptamers can be designed into analyte-responsive biosensors and thus improve detection sensitivity by reducing background [36–40] or amplifying signals [41–44]. The term DNAzyme refers to nucleic acids with certain sequences and structures that give them catalytic ability towards various chemical reactions, such as RNA or DNA cleavage, ligation and porphyrin metalation [45, 46]. The first DNAzyme was isolated by a selection technique involving catalysis of RNA phosphodiester bond cleavage in the presence of lead ion [47]. Similar to enzymes, DNAzymes often recruit transition metal ions as "cofactors" [48] to improve catalytic efficiency. Compared with enzymes, DNAzymes are much more stable at high temperatures, and they can be adapted for use in analytical sensors.

In addition, DNA is physically and chemically stable, biocompatible and easy to manipulate and modify, and it can be renatured after denaturing at elevated temperature. DNA can be modified with many functional groups, such as acrydite, amino, carboxyl and thiol, which can react with other functional moieties. With the development of phosphoramidite chemistry, most DNA modifications can be incorporated with the programming of a DNA synthesizer for easy preparation and accessibility.

DNA can be physically entrapped into hydrogel networks, or covalently conjugated to the polymer backbones. Chemical modification usually involves functionalization of DNA with an amine to react with a succinimidyl ester polymer/gel [8] or by use of acrydite DNA to polymerize with free acrylamide monomers [49]. In some hydrogels, the DNAs are immobilized inside the permanently crosslinked network, while in other cases, the DNA strands are used to crosslink the polymer chains. Moreover, DNA by itself can form hydrogels with help from DNA ligases [50]. In this review, we will focus on DNA hydrogels that respond to various stimuli, as well as the current applications of these DNA-incorporated smart hydrogels.

Response of DNA hydrogels to different stimuli

In DNA hydrogels, either a change in environmental conditions or occurrence of an external stimulus changes the DNA structure, which, in turn, affects the properties of the hydrogel. The changes in DNA include hybridization/dehybridization of the DNA double helix or a conformational change of ssDNA. Hydrogel properties which are affected can be phase, volume, optical properties, or surface properties. This section briefly reviews DNA hydrogel response to different stimuli.

Temperature

Temperature is one of the most common of all external stimuli. Thermosensitive hydrogels usually show a reversible phase transition at a critical solution temperature. For instance, Tanaka reported the collapse of a polyacrylamide hydrogel upon lowering the temperature [51]. DNA hydrogels can be thermoresponsive as well. The design takes advantage of the hybridization between complementary base pairs of DNA and the thermal dissociation characteristics. The first thermoresponsive DNA hydrogel was made from succinimide copolymers [8]. The succinimidyl ester group covalently immobilizes 5′-terminal-amino-modified DNAs to the side chains of a linear polymer, and the resulting copolymers remained in the solution phase (sol). When the crosslinker DNAs, i.e., DNA strands that were complementary to the polymer-DNA, were added to the solution, they hybridized with polymer-DNAs and transformed the linear polymers into a gel state (gel). The hybridization occurred at room temperature and was stable below the DNA dehybridization temperature, called the DNA melting temperature, or Tm. At temperatures above T, the double-stranded DNA became unstable, and dehybridization led to gel dissolution (Figure 1a). The gel reversibly transitioned between the gel and sol states. This work demonstrated a synthetic method for DNA-grafted polymer materials and reported a novel hydrogel with DNA as crosslinkers.

Another interesting thermosensitive DNA-hydrogel was recently made by Kang et al. [52]. In this work, gold-silver-based nanorods (NRs) were used as templates for colloid- based polymerization. The methacryl groups on the NR surface could initiate the growth of linear polymeric chains through copolymerization with the acrylamide monomers, some of which were modified with oligonucleotides. NR-anchored, DNA-grafted polyacrylamide polymers were crosslinked by a complementary ssDNA sequence that could hybridize with acrydite-modified DNA to form a core-shell nanogel. The encapsulated NR core absorbed near infrared (NIR) photon energy and converted it to heat with high efficiency. When irradiated by a NIR laser beam, the NR elevated the temperature of the surrounding gel. When the temperature was higher than the DNA Tm, double-stranded DNA dissociated, and the gel collapsed. The authors also demonstrated gel loading with an anticancer drug for controlled released.

In the previous examples, DNA hydrogels formed at low temperature and dissociated at high temperature by the dehybridization of double-stranded DNA. In one study, Xu et al. described DNA hydrogel formation at high temperature [53]. The gel was prepared by mixing double-stranded DNAs with graphene in solution followed by heating to 90°C for 5 min. At high temperatures, double-stranded DNA was unwound to ssDNA, which could bridge adjacent graphene sheets via π-π stacking. The formed hydrogel was stable at room temperature for one week.

Ions (pH)

Ions, including protons and metal ions, have been observed to induce DNA conformational changes. The concentration of protons, measured as the pH, can influence the formation of a cytosine-rich DNA [54]. Some metal ions are recruited as “cofactors” in DNAyzme function. These special DNA structures have been incorporated into DNA hydrogels to make them

responsive to ions. Since ions are smaller than molecular species, they can diffuse more rapidly inside hydrogels, leading accelerated response time.

Hydrogels responsive to protons, also called pH-sensitive hydrogels, are usually formed by polymers with weakly acidic or basic pendant groups, which can accept or release protons according to the change in environmental pH. Cheng et al. designed a pure DNA hydrogel responsive to pH [55]. First, three DNA strands were designed to form a three-armed DNA nanostructure (Y unit). At a high pH, Y units remained isolated in random coils because of their electrostatic repulsion. When the pH dropped to slightly below 7, the cytosines became partially protonated, which led to the crosslinking of Y units into i-motif structures. Gold nanoparticles (AuNP) introduced before the gelling transition were trapped inside the hydrogel to serve as tracer agents. Addition of base quickly disassembled the gel and released the trapped particles into solution. The gel-to-sol transition occurred less than a minute after the pH was increased from 5 to 8, indicating a fast response. The authors suggested that because tumors and sites of infection are usually more acidic than other parts of human body, this pH-sensitive hydrogel could be used for responsive drug release. Based on this work, Cheng et al. constructed a pH-responsive DNA-SWNT (single-walled carbon nanotube) hybrid hydrogel [56], which has DNA duplex linear units with cytosine-rich sticky domains at each end and SWNTs wrapped with specially designed DNA structures as crosslinking units.

Hydrogels responsive to metal ions included functional DNA motifs, such as aptamers or DNAzymes. One design used Cu^{2+} DNAzyme/Substrate complexes as crosslinkers to assemble DNA-grafted polymers. Two strands of DNA, DNAzyme and Substrate were incorporated into two linear polyacrylamide polymers, respectively, and mixed to form a 3D hydrogel via the hybridization of the complementary DNAzyme and Substrate sequences. In the presence of Cu^{2+}, the Substrate sequence was irreversibly cleaved at the cleavage site (Figure 1b). This destabilized the DNA duplex and led to the dissociation of two DNA side-chain polymers, finally transforming the hydrogel into a liquid form [57]. This system is highly selective to Cu^{2+} and showed a fairly rapid response. Other than copper, DNAzymes specific to other ions, such as Pb^{2+}, can be adapted in this system.

Another design incorporated DNA aptamers into the hydrogel network and produced a color change of the hydrogel upon target recognition. Mercury ions interacted with thymine base pairs to help DNA form a stable hairpin structure. A DNA staining dye (SYBR Green I) could then bind to the hairpin and emit green fluorescence. This hydrogel selectively removed Hg^{2+} from buffers and environmental water samples. Although binding to ions induced an aptameric conformational change, the hydrogel did not experience a phase transition with target ion addition. This is because the immobilized aptamers were not involved in hydrogel crosslinking, making this design different from the previous DNAzyme-based hydrogel.

Biomolecules

Biochemically responsive hydrogels have attracted increasing attention because of their applications in biomedical fields. Molecular recognition moieties, such as antibodies [5] and enzymes [58, 59], have been integrated into hydrogels, largely expanding the range of stimuli

compared to conventional hydrogels. Similarly, DNA probes, i.e., aptamers that recognize biomolecules, can be pre-trapped inside hydrogels to further diversify the available stimuli. The design is based on the competitive binding of targets to the trapped DNA aptamers. The first report was a hydrogel response to adenosine [60] (Figure 2a). First, two single-stranded DNA (ssDNA) were covalently conjugated to acrydite and copolymerized with acrylamide, respectively. A linker DNA capable of hybridizing with the two acrydite-modified DNA sequences was used to link the two polymers to form a rigid hydrogel. The linker DNA also contained an adenosine-binding aptamer sequence that was partially hybridized with the polymer-DNA. In the presence of target adenosine molecules, the aptamers competitively bound to targets and left only five base pairs to hybridize with one of the strands in the hydrogel. Thus, the gel became unstable and converted to solution phase within 15 minutes. The aptamer-gel system was very selective for adenosine; addition of cytidine, uridine or guanosine at high concentrations did not cause gel-to-sol transition. Also, when mutated aptamer strands were used, the gel no longer responded to adenosine. To demonstrate the generality of the method, a thrombin-binding aptamer was tested, and similar phenomena were observed. However, the thrombin-induced phase change was much slower, possibly resulting from the slow diffusion of thrombin in the gel. This principle was adapted to detect small molecules with improved sensitivity [61]. For example, less than 20 ng of cocaine can be detected with the naked eye within 10 min by using this simple enzyme-trapped aptamer cross- linked hydrogel.

Another strategy employs allosterically regulated DNA-binding proteins that bind to conserved DNA motifs and dissociate in the presence of allosteric molecules. The proof-of-principle work was demonstrated by Christen et al. with the tetracycline repressor (TetR) protein and its cognate *tetO* DNA operator [62] (Figure 2b). The hydrogel was constructed with two types of linear polyacrylamide: one electrostatically coupled to TetR proteins and the other covalently functionalized with *tetO* DNAs. The polymer- immobilized TetR protein was engineered to a single-chain variant to prevent hydrogel formation via TetR dimerization. In addition, a hexahistidine tag was added to the C-terminus of the protein to interact with Ni^{2+}-functionalized polymer. The interaction between TetR and *tetO* crosslinked these two polymers and resulted in hydrogel formation, while the addition of tetracycline caused the dissolution of hydrogel within 2 hours. Using Interleukin-4 (IL-4) as a model drug, the authors demonstrated the release of preloaded IL-4 from the tetracycline-responsive hydrogel in a dose-dependent manner. The bioactivity of the released IL-4 was comparable to the IL-4 standard, indicating the biocompatibility of the hydrogel.

It is well known that phosphodiester linkages in the DNA backbone can be hydrolytically cleaved by deoxyribonucleases (DNase). Therefore, DNA hydrogels can also respond to DNase treatment. Xing et al. reported a self-assembled DNA hydroge that showed enzymatic responsiveness [63]. The authors designed a Y-scaffold and a linker DNA structures that could hybridize with each other and form a hydrogel. When an enzymatic restriction cleavage site was inserted in the linker sequences, the DNA hydrogel underwent a gel-to-sol transition upon addition of the corresponding restriction enzyme.

DNA

DNA sequences can also be used as stimuli. For example, DNA can reversibly control the gel-sol transition, induce changes in mechanical properties, or produce volume change. Lin et al. induced a gel-sol transition by competitive replacement of crosslinking

DNAs with free DNA sequences [64]. The hydrogel was formed by a ssDNA hybridized with two polymer-DNA sequences. In addition, the crosslinker DNA contained a toehold region to initiate hybridization with a removal DNA strand. The displacement of the shorter-length hybridized sequence with a longer-length one via a three-way branch migration is thermally driven [65], and the design is being used in several DNA-based molecular machines [66–68]. Therefore, the hydrogel was dissociated upon the formation of a double strand between the crosslinker and the removal DNA.

Later, the same group reported a reversible change in the stiffness of a hydrogel by DNA strands [69]. They synthesized a hydrogel with ssDNA as crosslinkers that covalently attached to two polyacrylamide chains. The flexible ssDNA structure made the gel less stiff. Then a fuel strand with a base sequence complementary to the ssDNA plus a toehold was added. It hybridized with the crosslinker ssDNA, stiffened the hydrogel, and caused a tensile force along the polymer chains. The addition of the removal strand perfectly complementary to the fuel strand led to competitive binding, converting the crosslinker DNA back into ssDNA and transforming the gel back into its initial flexible state. Thus, the changes in reversible stiffness were attributed to the fuel and removal strands. Gel swelling, electrophoretic compression, and changes in microstructure are properties affected by this change.

By the same principle, DNA can cause a hydrogel to shrink or swell. Murakami et al. first reported hydrogel shrinkage upon addition of ssDNAs in 2005 [70]. In their work, they examined hydrogels containing ssDNA conjugated to polyacrylamide by direct grafting and by forming semi-interpenetrating polymer networks (SIPN). They showed that these two types of gels contracted in response to complementary ssDNA, but not to DNA having a one-base mismatch. The authors hypothesized that the volume change was caused by dehydration following the formation of double-stranded DNA. Later in that year, the same group reported a hydrogel capable of both shrinking and swelling in response to specific DNAs [71] (Figure 2c). This hydrogel was synthesized by polymerization of acrylamide with 5′- and 3′-methacryloyl-modified ssDNA as the crosslinker. Therefore, the gel was permanently crosslinked by ssDNAs. The volume change of a hydrogel was achieved by varying the sequence and conformation of ssDNA crosslinker in the hydrogel. When the crosslinker DNA contained no intramolecular base pair to form any confined secondary structure, the addition of its complementary strand (cDNA) led to formation of a double helix with the crosslinker, which was observed to shrink the gel by dehydration, as previously discussed [70]. When the crosslinker DNA was designed to form a molecular beacon structure, the hybridization with cDNA opened the loop structure and extended the ssDNA, causing the gel to swell. Although the authors did not show how to convert the volume change of the gel into practical use, this study indicated the potential of DNA-hydrogels in engineering actuators.

Photons

Inspired by the DNA-responsive hydrogels, photoresponsive DNA hydrogels were reported with a modification of DNA molecules. The photosensitive molecule azobenzene (Azo) isomerizes between the trans and cis states, depending on UV-visible light irradiation, which, in turn, determines the hybridization between Azo-modified DNA and its cDNA. In one study, Kang et al. incorporated Azo into the backbone of crosslinker DNA sequences [72] (Figure 2d). Under visible light, the Azo molecule was in the trans form and allowed crosslinker DNA to hybridize with DNAs on the polymer side chains, thereby forming a 3D hydrogel network. When the gel was irradiated with UV light, the Azo was photoisomerized to the cis form that prevented hybridization and caused the hydrogel to revert to the sol state. They demonstrated that the gel-sol conversion was reversible by alternating irradiation between vis and UV light. In another work, Lu et al. demonstrated a photocontrollable hydrogel volume change [73]. Azobenzene-tethered ssDNA and its cDNA were grafted into a polyacrylamide hydrogel network. The gel was permanently crosslinked by N,N-methylenebisacrylamide, as well as reversibly by tethered ssDNAs. The hybridization between the two ssDNAs could be manipulated by alternating irradiation of UV and visible light, which was attributed to the conformational change of azobenzene. The formation of DNA duplex crosslinkers resulted in shrinkage of the hydrogel, while dissociation caused the hydrogels to expand. The change in molecular level was reflected in the microscopic volume of the hydrogel. A light-responsive dynamic hydrogel capable of harvesting light energy into a macroscopic volume change may be used to fabricate actuator devices for different applications.

Applications of responsive DNA hydrogels

Since DNA hydrogels can respond to different kinds of stimuli, they have been employed in various applications, especially in sensing, capture and controlled release. This section reviews the current biological applications of DNA hydrogels.

Sensing

To act as a sensor, two features are required: a recognition element to sense the analyte and a reporting system to transduce signals. Stimulus-responsive DNA hydrogels meet these criteria, and several sensing modalities have been integrated into the property changes of hydrogels.

When the analyte is capable of inducing a gel-to-sol conversion, gold nanoparticles (AuNP) are often used to visualize the transition. AuNPs have a large extinction coefficient in the visible region, and the characteristic red color makes them a sensitive indicator for visual detection. In addition, water-soluble AuNPs with different diameters can be easily synthesized for use in hydrogels with different pore sizes. In general, AuNPs are trapped inside a hydrogel during gelation, giving the gel a red color (Figure 1c). Addition of a buffer solution on top of the gel does not change the shape of the gel, and there is a clear separation line between the red hydrogel and colorless solution. Entrapped AuNPs can stay inside the gel for several hours without leaking [55]. Addition of the target molecules to the system disrupts the network, triggering the gel-to- sol transition and releasing AuNPs from the gel

to the upper layer buffer solution. The distinct phase difference gradually disappears because the red AuNPs diffuse into the previously colorless buffer solution, as detected by measuring the absorbance at 520 nM or visually by the naked eye. This system has been applied in sensing pH change [55], as well as the detection of metal ions [57] and small molecules [60]. Detection limits are usually in the micromolar range, precluding detection of trace amounts of analyte. The kinetics depends on the size of the targets, with larger sizes diffusing more slowly in the gel and causing prolonged release. For example, as described above, the release process of the thrombin-induced AuNPs is slower than the adenosine-induced release [60].

Based on the AuNP-diffusion system, Zhu et al. introduced an enzyme into the gel system to realize a sensitive, simple and cost-effective visual detection of cocaine [61] (Figure 3a). Instead of AuNPs, amylase, which hydrolyzes amylose to maltose, was trapped in the DNA-grafted polyacrylamide gel. In the buffer solution, amylose with iodine yielded a dark blue color. Because both amylase and amylose are large polymers, they were physically separated by the hydrogel. The crosslinker DNA was a cocaine- binding aptamer that could be competitively removed from the gel by cocaine. Therefore, in the presence of cocaine, the gel broke and released amylase to the buffer solution. Amylose was then digested by amylase, and the dark blue color disappeared. One important feature of this detection is the use of an enzyme for signal amplification. As long as sufficient amylase was released to the solution to cause the color change, it was not necessary to have the entire gel completely dissolved. The authors demonstrated that this platform could detect less than 20ng cocaine in 10 min with the naked eye. To perform a quantitative analysis, the same group introduced a glucose meter to the hydrogel detection system [74]. The design of hydrogel was adapted from the above visual detection system. In this case, glucoamylase that hydrolyzes amylose to glucose was stably encapsulated in the hydrogel, while the substrate amylose was in the solution outside the gel. In the presence of target molecules, aptamers preferentially bound to the targets, leading to decomposition of the hydrogel and release of glucoamylase. Therefore, the nonglucose target recognition event was efficiently converted into a cascaded glucose production reaction, which could be sensitively and quantitatively detected by the glucose meter. The authors reported a detection limit of 3.8 μM of cocaine in buffer solution, 4.4 μM in urine, and 7.7 μM in the presence of human plasma, all of which were in the same range, indicating the applicability of this method in complex samples. The hydrogel/glucose meter platform is accurate in comparison to the standard LC/MS method for cocaine detection [74], and it is widely applicable to a range of nonglucose targets with different aptamers. Although the limit of detection (LOD) is relatively high, this hydrogel detection method is inexpensive, rapid, portable and quantitative.

Another type of detection utilizes gel-immobilized DNA to induce a color change of the gel upon recognition of the target. Most hydrogels are colorless and transparent, thereby assuring a low optical background in detection. In addition, immobilized probes allow washing steps and signal amplification to improve sensitivity. Moreover, sensitivity can be enhanced by entrapping a large number of chromophores in a three-dimensional volume, as opposed to a two-dimensional surface [75].

Baeissa et al. used acrydite-modified DNA, which was covalently conjugated to the polyacrylamide hydrogel, to detect target DNA [76] (Figure 3b). In this design, another probe DNA was conjugated to AuNPs as a colorimetric sensor. Without the acrydite DNA, the optical background of the gel was not detectable, indicating a very small nonspecific interaction between AuNPs and the gel matrix. The target DNA linked the AuNPs to the hydrogel to give a red-colored gel surface. The authors optimized the acrydite DNA concentration and the hydrogel percentage to reach a detection limit of 0.1 nM target DNA, which is comparable to that of other surface detection methods. The LOD could be improved to 1 pM with a silver enhancer kit. The signal amplification was realized by the deposition of silver metal on AuNPs surfaces in the presence of silver ion and a reducing reagent. The silver-coated AuNPs could grow into micrometer-sized particles with a higher contrast signal compared with bare AuNPs. The authors also tested selectivity with a target DNA carrying a single-base mismatch. Although the mismatched DNA showed a similar red-colored conversion of the gel at room temperature, the Tm was at least 15°C lower than that of the perfectly matched DNA target. Therefore, the selectivity could be realized by raising the gel temperature to a point where mismatched DNAs were dissociated, while the perfectly matched target DNAs were maintained. The detection system could be recycled by a simple thermal denaturation step to remove AuNPs-DNA and target DNA from the gel.

Besides AuNPs, fluorescent molecules, including DNA staining dyes, have been used to transduce optical signals. For example, a dye molecule known as SYBR Green I produces a yellow fluorescence when bound to unfolded DNA, but it emits green fluorescence when bound to hairpin DNA. Dave et al. used this color change to detect Hg^{2+} with a hydrogel-immobilized mercury-binding DNA [77] (Figure 3c). The mercury ions induced thymine-rich DNA to form a hairpin structure, resulting in a color change from yellow to green. The detection limit was as low as 10 nM. Later, the same group reported that introduction of 20% positively charged allylamine monomer into the cationic or neutral polyacrylamide gel lowered the background yellow fluorescence as a result of the repulsion between the positively charged dye and the gel backbone [78]. The signal-to-noise ratio was greatly improved, and the detection limit reached 1.1 nM. Similarly, guanine-rich DNA that formed a quadruplex with Pb^{2+} was immobilized into a hydrogel for lead ion detection [79]. Thiazole orange (TO) dye stained ssDNA with a yellow fluorescence. In the presence of Pb^{2+}, two adjacent DNAs assembled to form a quadruplex such that TO produced a green, instead of yellow, fluorescence. Pb^{2+} could be visually detected down to 20 nM. The authors finally demonstrated simultaneous detection of both Pb^{2+} and Hg^{2+} in the same water sample with hydrogels in different shapes.

In another example, a fluorescein-labeled ATP aptamer with a dabcyl-labeled cDNA were both entrapped within a hydrogel to sense ATP [36]. The fluorescence from the ATP-binding aptamer was initially quenched by the close proximity to the dabcyl, a quencher molecule, which was conjugated to a short piece of DNA partially complementary to the aptamer. When ATP was added, it competitively bound to the aptamer, inducing a conformational change, thereby displacing the short cDNA sequences. Removal of the quencher resulted in restoration of the fluorescent signals of the fluorescein, and the hydrogel glowed.

Volume change is an important feature of some responsive hydrogels. However, the degree of swelling or shrinking is usually difficult to measure and relate to the amount of stimulus. To address this problem, several approaches have been tested. In one attempt, Tierney placed the DNA hydrogel over the cross section of an optical fiber to capture the small volume change [80] (Figure 3d). Two ssDNA grafted in the hydrogels could partially hybridize with each other, leaving a flank region on each strand. The target DNA destabilized the double helix by competitive hybridization with one strand, causing the hydrogel to swell. The interference of light guided by the optical fiber and reflected at the fiber-gel and gel-solution interfaces enabled optical detection of the optical path length within the gel. Changes in physical length and refractive index of the gel changed the optical path length within the gel, which was detected and correlated with the volume change of the gel. They reported a resolution of 2 nm, which enabled the detection of 500 nM DNA.

A second example involved monitoring of the Bragg diffraction of DNA- responsive hydrogel beads for multiplex label-free DNA detection [81]. The hydrogel beads were fabricated by template replication of silica colloidal crystal beads to obtain a photonic crystal structure. The structured DNA hydrogel could convert a slight physicochemical change into a quantitative spectral signal. Quantum dots (QD) were covalently attached to the hydrogel photonic beads for multiplex label-free DNA detection. The specific hybridization of target DNA with the QD-tagged probe DNA caused the hydrogel to shrink, and the volume change was detected as a corresponding blue shift in the Bragg diffraction peak position of the beads. This method could quantitatively detect DNA with a LOD at 1 nM, which is lower than that of other label- free DNA detection methods.

Separation and purification

DNA hydrogels can also be used as separation platforms for target molecule capture or purification. Compared with traditional methods, DNA hydrogels can be applied to the separation of various targets, ranging from small molecules to biomolecules, and even whole cells.

One type of design takes advantage of the gel-sol phase change to capture the target of interest inside the gel. He et al. demonstrated the separation of ATP from a complex mixture using this principle [82] (Figure 4a). In this study, DNA-modified acrydites were copolymerized with acrylamide monomers to obtain linear polyacrylamide polymers with DNA side chains. The grafted DNA strands were crosslinked by a sequence with the ATP-binding aptamer motif. When incubated with the sample, aptamers bound to ATP to form a hydrogel with DNA-bearing polyacrylamide polymers. Thus, ATPs were captured inside the hydrogel, while nontarget molecules were excluded from the hydrogel after washing. ATP could be released from the hydrogel by cDNA displacement. The authors proved that the specificity and capture efficiency were not altered in the presence of nontarget molecules, such as GTP. Over 80% of the original quantity of ATP could be recovered using this hydrogel. They also tested thrombin protein to evaluate the generality of the system. The same concept was used to remove mercury from water by another group [77]. The polyacrylamide in the DNA-functionalized hydrogel can absorb mercury ions from water via the amide nitrogen at a rate of ~1 h^{-1}.

The porous feature of hydrogels makes them attractive substrates to immobilize affinity probes for target capture and separation. It has been shown that nucleic acid hybridization in gels closely resembles that in solution [83], which ensures thermodynamically stable hybridization that might otherwise be affected on solid surface supports [84]. Therefore, DNA hydrogels have been developed into nucleic acid hybridization assays. In a work reported by Olsen et al., a DNA-grafted polyacrylamide hydrogel was formed into microfluidic plugs [85]. The microfluidic plugs were fabricated by photopolymerization of acrylamide-modified DNA probes with a polyacrylamide matrix in polycarbonate microfluidic channels. Solutions containing fluorescently tagged cDNAs were electrophoresed into the plugs and then hybridized with polymer-DNAs. The captured DNA could be collected after electrophoretic or chemical removal from the hydrogel plug, thereby making the gel reusable. The authors demonstrated that the gel could be used to concentrate desired DNA strands from a diluted sample, as well as detect multiple DNA targets. Although the authors demonstrated both the capture and detection of DNA with the hydrogel plugs, the detection was based on the different fluorescent molecules pretagged on target DNAs, which is not very practical with real samples. Nevertheless, the incorporation of a hydrogel into a microfluidic device enables the fabrication of an automated DNA detection/capture platform. The analysis of nucleic acids by hydrogel-based microfluidics has been extensively studied and reviewed elsewhere [86, 87]. In addition to microfluidic plugs, DNA hydrogels were formed into other shapes, such as microparticles, for facile nucleic acid sensing/capture [88]. Lewis et al. used a replica molding technique to fabricate ssDNA modified poly-(ethylene glycol) (PEG) microparticles. Furthermore, a series of important parameters, such as humidity and concentration of PEG-diacrylate, probe DNA and photoinitiator were found to affect the formation of the DNA hydrogel microparticles.

Owing to pore-size restrictions, it is difficult to capture large entities like cells inside hydrogels. Therefore, monolithic hydrogels with DNA on the surface were developed to capture large targets. Zhang et al. tethered DNA aptamers on a hydrogel surface to capture cancer cells [89]. Aptamers specific to a cancer-cell membrane protein were immobilized on adaptor DNA sequences that were conjugated to the supporting hydrogel via free radical polymerization. After incubation with target cells, the hydrogel was observed to contain a total of 2519 ± 284 cells/mm. In contrast, only 6 ± 4 cells/mm control cells were detected, showing the specificity of the aptamer-tethered hydrogel. A third DNA sequence that was complementary to the aptamer sequence could displace the aptamer-captured cells, releasing aptamers and cells from the hydrogel surface so that the hydrogel could be reused for the next round of aptamer presentation and cell capture. The entire process took place under physiological conditions, and the capture and release controlled by nucleic acid hybridization was not destructive to the target cells, making downstream analysis or cell culture feasible. Later, the same group used the same hydrogel with directly immobilized aptamers on the surface to capture tumor cells. Instead of cDNA, endonuclease was used to remove captured cells from the hydrogel [90]

Controlled release

Temporal and spatial control of drug delivery improves the efficacy of the drug and also diminishes side effects. Hydrogels are made from hydrophilic synthetic or natural polymers,

with tunable pore sizes and stability. These characteristics make hydrogels one of the most appealing systems for controlled drug release. Pure DNA hydrogels, or those formed from biocompatible or biodegradable polymers, are suitable to load small-molecule drugs or biomacromolecules for sustained or target-responsive delivery.

Sustained release depends on the properties of a hydrogel, especially the pore size and degradation rate, as well as the diffusion rate of the entrapped molecules. Some small molecules that bind to oligonucleotides via noncovalent interactions, such as electrostatic interactions [91], minor or major groove-binding [92], or intercalation [93], have been incorporated into DNA hydrogels for controlled release. For example, Nishikawa et al. demonstrated a sustained delivery of doxorubicin, a widely used anticancer drug that can intercalate between base pairs of DNA with a pure DNA hydrogel [94]. Because the hydrogel was assembled from X-shaped DNAs by ligation, it was highly biocompatible and essentially nontoxic. The degradation of DNA after internalization by the cells also facilitated the release of doxorubicin. Moreover, DNA hydrogels can be immunologically active when incorporated with immunostimulatory motifs, such as unmethylated cytosine-phosphate-guanine dinucleotide. This may add extra value to the use of DNA for the delivery of anticancer drugs. The second example involves a DNA hydrogel for enhanced antibiotics loading with sustained release [95]. In this study, Zhang et al. reported that tetracycline loaded in the DNA-functionalized polyethylene hydrogel exhibited better antibacterial efficiency in comparison to the native hydrogel in *in vitro* antimicrobial experiments.

The controlled release of macromolecule drugs, such as proteins, from hydrogels can be assisted by affinity ligands, e.g., aptamers. By tuning the binding affinities of aptamers, Soontornworajit et al. demonstrated controlled protein release from an *in situ* injectable aptamer-functionalized hydrogel [96]. A previously selected aptamer that binds to platelet-derived growth factor B (PDGF-BB) [97] was altered by randomizing the nonessential nucleotide tail, or by mutating the essential nucleotides. The process generated a series of aptamers that bound to PDGF-BB with different dissociation constants. Aptamers were then functionalized to streptavidin-coated polystyrene particles and incubated with PDGF-BB to attach proteins on the particles. The protein-coated particles were then incubated with poloxamer (ethylene oxide and propylene oxide block copolymers) hydrogel at a temperature below the gelling temperature to entrap particles inside. The release of PDGF-BB was modulated not only by the dissolution of the poloxamer hydrogel, but also by the aptamer's affinity.

Target-responsive hydrogels can be used to precisely control release time and site. Using fluorescent semiconductor QDs as a model molecule, Liedl et al. studied a DNA- triggered release of QDs from a DNA-crosslinked polyacrylamide hydrogel [98]. Addition of the "release" strand of DNA unlinked the gel by removal of the crosslinker strands by branch migration. As a result, the gel dissolved, and the QDs diffused out of the gel. The diffusion behavior of the QDs in the gel and sol states was characterized using fluorescence correlation spectroscopy and fluorescent microscopy. The authors discovered that even particles with smaller diameters than the mean pore size of the gel were tightly trapped in the crosslinked gel. However, applications of such DNA-controlled release are limited by

the low concentration of naturally occurring nucleic acids in body fluids. To address this question, the authors mentioned that incorporation of aptamers or allosteric aptazymes could be an alternative to DNA control. Nevertheless, this work clearly demonstrated the feasibility of developing a DNA-hydrogel for a controlled release platform.

Another type of design engrafted biomolecules into the hydrogel with affinity probes and used cDNA to dissociate the gel and release the proteins. In one study, Wei et al. grafted two DNA strands on the main chains of polyacrylamide, which were crosslinked by a thrombin-binding aptamer in the presence of thrombin [99]. The crosslinking was achieved by extending the 5′ and 3′ ends of the aptamer with sequences complementary to the hydrogel DNA backbone, while leaving the aptamer sequence intact. Therefore, thrombin proteins were retained in the hydrogel by binding to the aptamers. Adding to the system a sequence fully complementary to the crosslinking DNA led to conversion to the sol state and release of the thrombin. As an alternative to direct binding of hydrogels to increase gel loading capacity, the proteins were loaded onto aptamer-coated streptavidin beads, which were then trapped inside an agarose hydrogel [100]. The protein could be released by adding DNA sequences complementary to aptamers (Figure 4b). This system was later designed for the controllable release of multiple protein drugs at desired time points [101].

The previously described hydrogel that functioned with allosterically regulated DNA-binding proteins has been designed for target-triggered drug release as well. In one case, Geraths et al. constructed a urate-responsive hydrogel that dissolves at pathological urate concentrations [102]. The hydrogel contained a *Deinococcus radiodurans*-derived urate repressor (HucR) that binds to its cognate operator sequence *hucO* at low urate concentrations, while it dissociates from the DNA sequence at elevated concentration. The HucR protein was coupled to polyacrylamides via an established method [62]. HucR- binding multimeric *hucO* DNA sequences were used to crosslink HucR-incorporated polymers. In the presence of pathological urate concentration, the authors demonstrated that the hydrogel gradually dissolved. However, at physiological level, the dissolution was strongly attenuated. Since elevated urate serum concentration is the cause of gouty arthritis, this hydrogel could be developed to load therapeutics that can lower urate concentration and simultaneously release them at a pathological urate level.

Controlled release can also be realized with hydrogels responsive to physical parameters. As previously described, light-[72] or temperature[52] -driven DNA hydrogels have been investigated for controlled release of doxorubicin. Although enhanced cell death was observed after UV irradiation of the light-sensitive hydrogel, clinical use is impractical as a result of toxicity and the low tissue penetration power of UV light. The temperature-sensitive hydrogel, on the other hand, utilized photothermal effect of Au-Ag NRs mediated by NIR irradiation. Compared with UV light, NIR can penetrate deeper into biological soft tissues without damaging them. Therefore, to control the release, NIR is safer and more effective than UV light. In addition, the hydrogel was functionalized with aptamers for specific cancer cell recognition. In this way, the encapsulated anticancer drug doxorubicin can be spatially and temporally released.

Conclusion and outlook

Hydrogels that respond to stimuli and undergo property changes are being intensively investigated for their potential applications in biosensing and biomedical research. As natural polymers, nucleic acids provide several unique features either alone or in combination with synthetic polymers. DNA-inspired hydrogels sensitive to temperature, ions, small molecules or specific DNA strands have been developed to expand the scope of available smart hydrogels. Stimulus-induced DNA hydrogel volume, color and phase changes have been observed and explored in the design of sensors, separation platforms and controlled release systems. In addition, it is noteworthy that the design of hydrogels for novel applications, such as DNA-based molecular logic gates [103104] and mechanical actuators [71, 73], have been attempted. Although these studies are still in the conceptual stage, they represent promising potential uses of DNA hydrogels.

Current studies have also revealed some challenges in the practical use of hydrogels. Although direct colorimetric visual detection based on hydrogel phase change is simple and effective, the LOD is usually high and cannot be quantified. Simple signal amplification mechanisms, such as the amylase/iodide system described above, should be explored together with the hydrogel design. Another concern in using hydrogels for detection is the diffusion time of the analyte within the gel. Slow diffusion may hamper the sensitivity and lengthen the detection time. It has been shown that response times depend heavily on the target molecule size. For example, hydrogels could sense pH change within a minute [55], while response to thrombin protein required hours [60]. Research to improve the response rate to biomolecules is therefore needed.

Biocompatibility and porosity, as well as programmability, make DNA hydrogels ideal materials for controlled drug delivery. Although different stimuli, including pH [55], light [72], heat [52], small molecules[102] and DNA strands [101], have been explored for the controlled release of drugs, most have only demonstrated the concepts in solutions or cell culture dishes. In-depth studies may find that some of these stimuli, e.g., UV irradiation and addition of DNA strands, are not feasible *in vivo*. The low pH at tumor and inflammation sites should be considered for triggering the drug release, but not for hydrogel formation. Other types of stimuli, such as magnetic fields, which can be applied to the human body, should be explored. To adapt hydrogels for clinical drug delivery, extensive studies, including *in vivo* evaluation, are needed to develop hydrogels for targeted and controlled delivery of current therapeutics, such as insulin.

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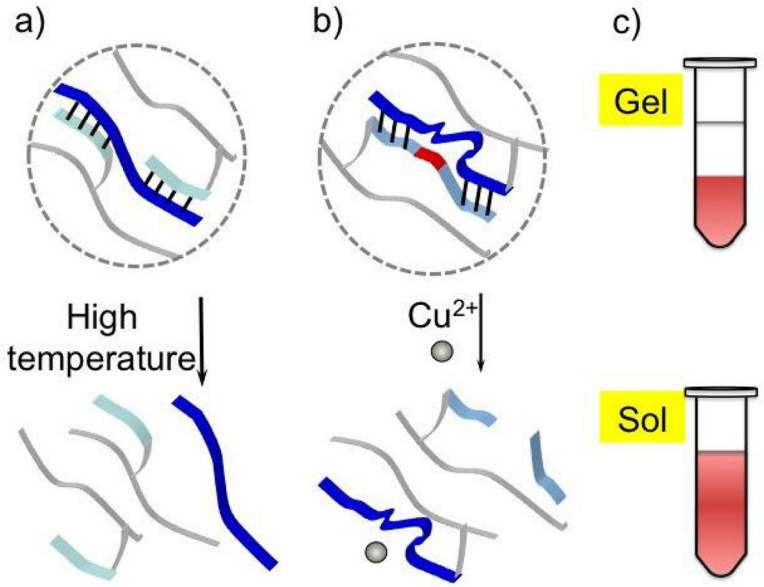

Figure 1.

DNA hydrogel response to temperature and ions. a) Hydrogel dissolution at high temperature. b) Metal ions induced DNAyzme activity, resulting in gel-to-sol transition. c) AuNPs entrapped inside the gel. The upper buffer solution is initially colorless, but when the gel dissolves, the AuNPs are released to the solution, giving it a uniform red color.

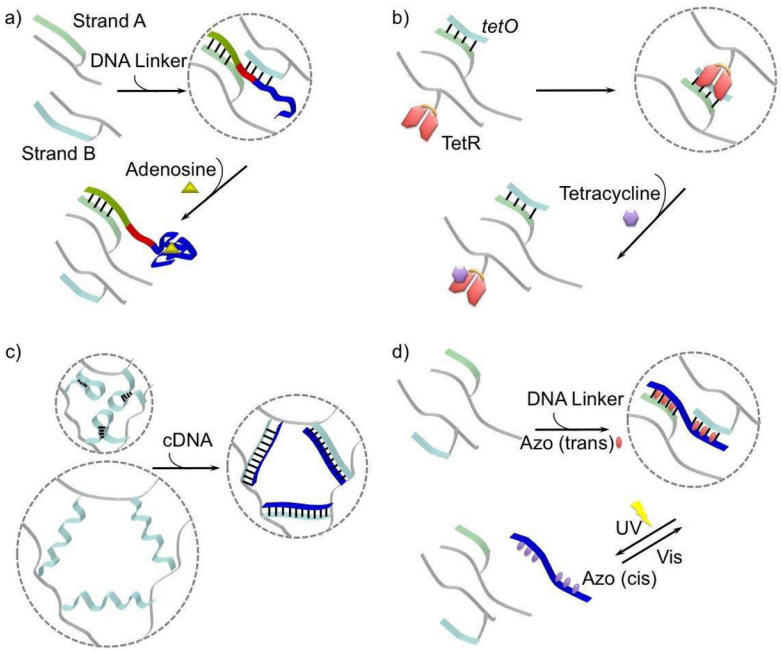

Figure 2.

DNA hydrogel response to molecules and photons. a) Hydrogel crosslinked by a DNA sequence containing adenosine-binding aptamer is converted to the sol phase by adenosine. b) Gel-to-sol transition induced by addition of the tetracycline. c) cDNA induced hydrogel volume change. d) Hydrogel phase transition regulated by UV/Vis irradiation.

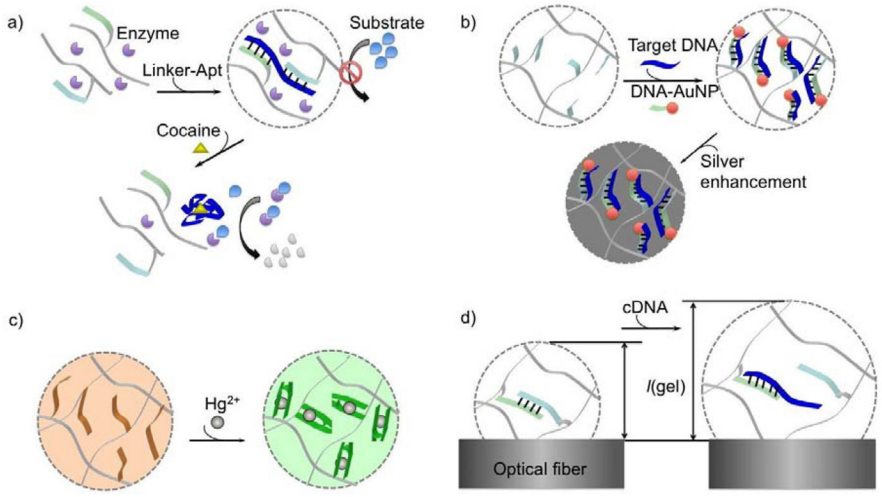

Figure 3.

DNA hydrogels as sensors. a) Visual detection of cocaine using a hydrogel with an enzymatic signal amplification step. Amylase is released only when cocaine is in the system, which can digest amylose and decrease the intensity of the blue amylose/iodine color. b) Transparent DNA hydrogel changes to red color in the presence of the target DNA. Addition of Ag^{+}, along with a reducing reagent, can increase the sensitivity of DNA detection. c) Detection of mecury ions with a DNA staining dye, SYBR Green I. The dye binds to extended ssDNA, producing a yellow color. When DNA folds into a hairpin structure in the presence of Hg^{2+} ions, the dye emits green fluorescence. d) Hydrogel volume change caused by DNA strands can be measured using an optical fiber.

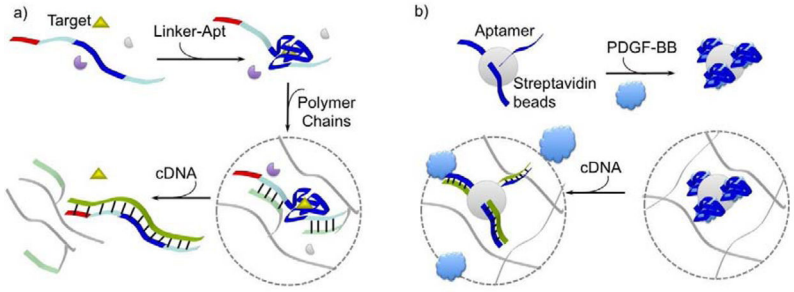

Figure 4.

DNA hydrogels for separation and controlled release. a) DNA hydrogels can be used to capture and purify small molecules. A linker DNA strand containing an aptamer moiety can capture target molecules. Addition of polymer-DNA forms a hydrogel with the linker DNA and traps target molecules inside hydrogel. After washing, target molecules can be released by cDNA strands. b) Proteins bound to aptamer-coated beads are trapped inside a hydrogel. Addition of cDNA can release proteins from the hydrogel.