DNA Hydrogel Assemblies: Bridging Synthesis Principles to Biomedical Applications

Mohammad-Ali Shahbazi, Tomás Bauleth-Ramos, and Hélder A. Santos*

DNA is a perfect polymeric molecule for interfacing biology with material science to construct hydrogels that represent fascinating properties for a wide variety of biomedical applications. Tunable multifunctionality, convenient programmability, adequate biocompatibility, biodegradability, capability of precise molecular recognition, and high versatility have made DNA an irreplaceable building block for the construction of novel 3D hydrogels. DNA can be used as the only component of a hydrogel, the backbone or a cross-linker that connects the main building blocks to form hybrid hydrogels through chemical reactions or physical entanglement. Responsive constructs of DNA with superior mechanical properties are very commonly reported nowadays, which can undergo macroscopic changes induced by various triggers, including alteration in ionic strength, temperature, and pH. These hydrogels can be prepared by various types of DNA building blocks, such as branched double-stranded DNA, single-stranded DNA, X-shaped DNA, or Y-shaped DNA through intermolecular i-motif structures, DNA hybridization, enzyme ligation, or enzyme polymerization. These hydrogels are envisioned for a variety of applications, such as drug delivery, sensing, tissue engineering, 3D cell culture, and providing template for nanoparticle synthesis. This review highlights the design of ideal DNA hydrogels from biological and material points of view for future biomedical applications.

1. Introduction

DNA is currently recognized as a major class of natural macromolecules in nature esteemed by scientists for designing unique functional materials. The last two decades have witnessed increased interest in DNA-based materials due to its many unique features and its recognition as one of the smartest natural polymers applied in medical science. The simplicity of molecular structure, desirable stability under physiological conditions held by stacking H-bonds and other weak forces, high density of backbone due to the existing phosphate groups, biocompatibility, as well as flexibility and reversibility, owing to the feasible breakage of base-pairs and strand separation, are among the main favorable attributes of these natural polymers.[1–3] Moreover, DNA has been attracting considerable attention as promising building block for robust and precise construction of predefined secondary structures due to the capability of sequence-directed hybridization.[4–6] These properties have directed scientists toward...
exceedingly versatile material systems due to their tunable properties and responsive behavior. Due to their unique characteristics, DNA hydrogels are of immense interest in the biopharmaceutical industry. Applications include the development of controlled drug delivery systems, diagnosis, and treatment of diseases such as cancer, diabetes, and cardiovascular diseases.

2. Physicochemical Features and Design Considerations of DNA Hydrogels

When thinking about the practical use of DNA hydrogels in biomedicine, the synthesized products must meet a number of biological and physical criteria depending on the intended application (Figure 1). For example, hydrogels designed for drug delivery must be able to sustain the release of cargos over time and the ones used for tissue engineering should be able to maintain loaded cells viable, mimic critical aspects of the natural extra-cellular matrix (ECM), act as a mechanical support, and degrade slowly in vivo to efficiently enable desired cellular functions. All these will be achieved through conscious selection of hydrogel components and synthesis method. In this section, we will address all considerations and key design criteria for producing DNA hydrogels.

3. Considerations of DNA Hydrogels

In this review, we first sought to comprehensively discuss physicochemical identifications and key features that can directly affect the design and synthesis of DNA-based hydrogels. Novel advances in the fabrication of DNA hydrogels and further recognition of unique properties that DNA can impart to the hydrogels are addressed next before summarizing the key design parameters to achieve responsive DNA hydrogels by reviewing the current studies reported in the literature. Moreover, in each section challenges and considerations are addressed. Next, potential usages of DNA hydrogels for different biomedical applications are highlighted by giving different examples of efforts intending to bring the molecule of DNA into the realm of bulk materials. Lastly, we presented a conjecture on the potential directions of such materials.
DNA hydrogels with favorable properties for defined biomedical applications.

2.1. Biocompatibility and Degradation Rate

Biocompatibility is the most critical parameter when considering the in vivo application of DNA hydrogels. It is a property of materials by virtue of which it affects the host tissue. In addition to low toxicity, DNA hydrogels must also be immune compatible to recognize any significant inflammatory responses elicited within in vivo microenvironments. In general, pure DNA hydrogels are biocompatible and biodegradable (since degradation products, i.e., nucleotides, are natural safe compounds), depending on the branched structure, concentration of DNA molecules, and the exposed microenvironment (e.g., in the absence or presence of nucleases). Considering the usage of different small molecules (e.g., monomers, polymers, initiators, and cross-linkers) for the formation of DNA hydrogels, the biocompatibility screening is essential to protect host body from possible adverse reactions caused by leachants in vivo. For example, unreacted maleimides used in Michael-type addition reactions are highly toxic on neurons or photoinitiators, such as 2,2-dimethoxy-2-phenyl-acetophenone can be cytotoxic within the host body.

Biodegradation rate of DNA hydrogels needs further exploration due to the lack of evidence and less attention to this important parameter. In drug delivery application, degradation rate of DNA hydrogel might be able to control the release of cargo molecules. For tissue engineering, a favorable degradation kinetic might allow cell proliferation and migration, or provide space for blood vessel infiltration. Ideally, to achieve real breakthrough, DNA hydrogels should be designed such that byproducts generated from degradation show high biocompatibility with no harsh inflammatory effect or eliciting foreign body responses.

2.2. Mechanical and Physicochemical Attributes

The success of DNA hydrogels implanted in the body will be mainly associated with their mechanical properties. It is well accepted that the mechanical properties of hydrogels can determine the efficacy of obtained responses from the loaded drugs or transplanted cells, that is, cell migration, proliferation, and differentiation. Depending on the fabrication method, DNA concentration, the stoichiometry of reactive groups, the density of cross-linker, and temperature are all commonly supposed to play a role in tuning the mechanical properties, viscosity, and gelation point of DNA hydrogels. In general, an optimum balance between mechanical properties, such as elasticity and matrix integrity, and degradation rate is vital to warrant proper function of DNA hydrogels within a desired timespan.

The adhesive property of hydrogels is of outmost importance when they are used for regenerative purposes. In many cases, hydrogels lack the adhesive property required for effective localization of transplanted cells in vivo. Therefore, developing a hydrogel with optimized adhesive properties can lead to improved therapeutic responses. Future studies need to focus on modification of hydrogels with adhesive functional ligands to promote their cell adhesive properties. This can be very crucial for efficient hydrogel-assisted cell transplantation.

Another important property of DNA hydrogels is their swelling capability in the aqueous conditions. This property might affect...
degradation rate of hydrogels and modulate the release kinetic of cargos from the gel. In a DNA-only gel system, swelling to over six times by volume has been reported in deionized water.[41] Costa et al.[41] have also investigated the swelling of plasmid DNA (pDNA) gels prepared by a cross-linking reaction with ethylene glycol diglycidyl ether (EGDE), revealing the dependency of swelling on the concentration of cross-linkers and ions in the solution. The next critical issue that should be taken into account is the size of pores in the hydrogels. Early exploration has primarily suggested pore size within the range of ∼20–100 μm is suitable for tissue engineering purposes,[42] however, smaller pores are needed for drug delivery applications in order to properly maintain payloads within the 3D structure of the gel. Therefore, the same principle might be applied for DNA hydrogels. In addition, pore interconnectivity, uniformity, and shape are needed to be investigated in DNA hydrogels as it might induce a significant impact in certain applications.

2.3. Stability of DNA Hydrogels

Although the response of DNA strand to temperature, pH, ion concentration, or enzyme allows the design of smart responsive hydrogels, it might also pose complications in maintaining the integrity of fabricated structures. This means that all the above-mentioned factors can contribute in the stability of the DNA hydrogels. For instance, ion containing buffers are better choice than water in maintaining hydrogel integrity and original structure. It has also been demonstrated that in DNA-only gel systems, gels produced by single-stranded DNA (ssDNA) possess less stability than those made of double-stranded DNA (dsDNA).[43] DNA cross-linked polymeric hydrogels also exhibited thermally dependent sol–gel transition due to the altered stability of the DNA base-pairs. Hence, designing DNA sequences to obtain desired melting temperature (Tm) is achievable by adjusting the length of DNA strand and G:C content since this base-pair is more stable than A:C due to the presence of stronger hydrogen bonds.[13,16] Besides, when designing, one should pay enough attention to stability of base components as the final hydrogel should not undergo any substantial functional changes during sterilization process.

2.4. Interactions at the Hydrogel–Biointerface

While plenty of efforts have been recently directed toward developing DNA hydrogels as a revolutionary implantable structure to sense body functions, facilitate drug delivery, or aid tissue engineering, one of the main challenges is how to mimic the dynamic nature of the ECM by DNA hydrogels. Therefore, parallel care needs to be exerted to recognize various strategies to control biological interactions at the material–cell interface as it can modulate the optimal function of surrounding cells and minimize the reactions of body’s immune system to the hydrogels. It is presumable that DNA hydrogels can get recognized as a foreign agent and encapsulate within a dense impermeable collagen capsule, which not only inhibit the function of the gel, but also can cause tissue distortion and pain.[44,45] Given that the ECM is a complex environment comprising a plethora of structural ECM proteins (e.g., collagen, fibronectin, laminin, and elastin), this phenomenon initially starts with nonspecific protein adsorption, followed by macrophage accumulation and secretion of inflammatory cytokines.[44,45] Since it has been shown to occur for diverse types of materials (hydrophilic, hydrophobic, hard, soft, polymeric, metallic, and ceramic) regardless of their nature, it seems very logical to speculate its probability for DNA hydrogels. In that sense, further studies should be performed in future to unveil the occurrence of these vexing interactions when DNA hydrogels are applied in vivo. This will open new areas of research in future to understand how to cope with interactions at the hydrogel–biointerface and improve the cross talk between the microenvironment and inserted hydrogels.[11,46,47] In general, it is supposed that the highly charged structure of DNA may potentially interact with an array of biological entities, such as protein, ions, and other small molecules, which can further change its biological, mechanical, and physicochemical properties. For example, it has been shown that mechanical properties of a DNA gel consisting of DNA strands of ≈2,000 bp can change by the exchange between mono- (e.g., Na⁺) and bivalent (e.g., Ca²⁺) cations under physiological ion concentration.[17] Such kinds of studies are essential for further rapid advancement in the logical design of DNA hydrogels.

2.5. Mass Transport

Considering the potential of DNA hydrogels in tissue engineering, verification of appropriate mass transport properties, similar to local surrounding tissues, is essential for proper functioning of encapsulated cells. This will help longer surveillance and proliferation of the cells through continuous exchange of proteins, nutrients, gases (i.e., O₂ and CO₂), and waste products into, out of, or within the hydrogel. In contrast, when applied for controlled delivery of therapeutics, initial burst release of the cargo is undesirable, therefore, restricted permeability of hydrogel matrix and controlled diffusion are essential parameters. These properties are highly correlated with the mechanical properties of the hydrogels network.[48,49] Therefore mass transport behavior is expected to be adjustable through the manipulation of the synthesis protocol, DNA length and concentration, or physicochemical properties of co-materials applied for the synthesis of hydrogels when DNA is used as a cross-linker or as a backbone. For example, amount of EGDE cross-linker was reported as an important influencing factor on mechanical properties and subsequently mass transport of the gels made of DNA backbone.[50]

3. DNA Hydrogel Fabrication: The Only Network, a Backbone or a Cross-Linker

Tending toward advanced fabrication of materials has opened new notions and thoughts to create various DNA-based structures with specified and predefined functions using cutting-edge approaches.[33,46] As a result of the progresses in chemical synthesis and purification technologies, DNA can nowadays be
synthesized and extracted in a large quantity and with high quality using an economically affordable manner.\textsuperscript{[51–54]} DNA can be served as the only network, building backbone, or versatile linkages for the synthesis of hydrogels.\textsuperscript{[5,21,23,28,41,47]} When applied as the only network, DNA gelation occurs based on physical interactions rather than chemical bonds. Owing to the high availability of the natural long dsDNA, this approach has been commonly reported despite bottlenecks associated with poor stability and lack of control over hydrogel formation at molecular level, which limit their scale-up application.\textsuperscript{[6,13]} It has been demonstrated that the swelling, mechanical characteristics, and biodegradability of DNA-only hydrogels can be dependent on the initial concentration and the conformational forms of building blocks.\textsuperscript{[52]} DNA-only supramolecular hydrogels were first reported in 2009 by Liu et al., who used 37-mer DNA strands for the gel formation.\textsuperscript{[53]} They exploited three partially complementary sequences at slightly basic pH to assemble a Y-shaped DNA structure comprised of a rigid double-stranded central domain and three ends of half-i-motif sequences as interlocking domains. By pH decrease to 5, the Y-shaped DNA structures connect to each other via i-motif structures to form 3D DNA hydrogel. Reversibility of i-motif structures at different pH allows hydrogel-solution transition by pH adjustment. In general, DNA hydrogels prepared by this method do not swell and lack efficient capability for encapsulating small molecules. This is mainly owing to the intrinsically rigid characteristic of duplex and i-motif structures as cross-linking points of the DNA hydrogel, limiting changes in the distance between connection points, that is, no flexibility and swelling.\textsuperscript{[55]} These hydrogels are mainly fabricated by ligase as a DNA conjugating enzyme or via base interactions by designing DNA strands composed of C-rich domains that facilitate high hydrogen bond formation between building blocks. Nevertheless, Lee et al.\textsuperscript{[56]} have used another approach where a polymerase enzyme was used to elongate DNA chains that can be noncovalently weaved as building blocks of hydrogel (Figure 2A). In this work, instead of ligase-mediated covalent cross-linkage of DNA chains and formation of chemical hydrogels, Φ29 polymerase (a bacteria phage polymerase) was used to form physical hydrogels which are composed of noncovalently woven DNA strands prepared in a two-sequential process. First, rolling circle amplification (RCA, or R) was performed for 4 h, followed by a 16 h multi-primed chain amplification (MCA, or M). The resulting meta-hydrogel showed solid-like properties when in water and liquid-like properties when taken out of water. Interestingly, the produced hydrogel possessed unusual mechanical properties of metamaterials and was able to rapidly reform its original shape in water after complete shape deformation in the liquid-like state (Figure 2B). While the potential of this meta-hydrogels as a flexible electric circuit has been tested, the authors have suggested the future capability of this gel in cell therapy and drug release. Similar synthesis strategy is recently reported for the fabrication a multifunctional DNA hydrogel with antibacterial and fluorescent functions.\textsuperscript{[57]} The synthesis route included three essential steps, including preparation of a circular DNA as the template for RCA, the elongation of DNA chain via an enzymatic polymerization using RCA, and the in situ formation of silver nanoclusters in RCA gel. In addition to RCA reaction, loop-mediated isothermal amplification\textsuperscript{[58–60]} and hybridization chain reaction\textsuperscript{[61,62]} have also exhibited favorable efficiency in the production of high dense pure DNA, which can be used for the fabrication of DNA-only bulk hydrogels.

While many of the previously reported approaches have used branched DNA motifs or circular DNA for the formation of DNA-only hydrogels,\textsuperscript{[6,13,27,29,63,64]} more recent attempts have shown the feasibility of generating DNA-only hydrogels by physical approach using linear dsDNA equipped with sticky ends. As an example shown in Figure 2,\textsuperscript{[65]} hybridization of two oligomers (O1 and O2) constructs a monomeric dsDNA building block O1-O2 with 30 base-pairs. Further equipment of this building block with two complementary sticky ends of 15 bases results in the self-assembly of hydrogels (Figure 2C1). The same protocol was followed to create O1-O3 monomeric dsDNA building block without noncomplementary sticky overhangs and showed no hydrogel self-assembly (Figure 2C2). Linear structures assembled by this strategy might have very high flexibility and low stability due to the noncovalent physical linkage and presence of nicks in the sugar–phosphate backbone after each ssDNA repeating unit.

As the backbone of hydrogels, DNA strands must be connected using cross-linking molecules or polymers via either chemical or physical (mainly electrostatic entanglement) interactions. For example, as a traditional method, chemically cross-linked DNA backbone can be produced through amine-epoxide reaction using EGDE.\textsuperscript{[58]} This approach is highly efficient for covalent cross-linking of the DNA chains regardless of their sequence contents. In addition, the physical and mechanical properties of the hydrogels can be tailored by adjusting the concentration ration of the DNA and cross-linker. Ionic interaction between DNA and surfactant poly-(vinyl)pyrrolidone\textsuperscript{[66]} or CTAB\textsuperscript{[67]} are reported as examples of physically formed gels containing DNA backbone. In an interesting study, a hybrid DNA-based hydrogel was fabricated via a programmable assembly approach using protein as conjugating reagent.\textsuperscript{[68]} This hierarchical hydrogel served as a biomimetic physiologic network and made based on supersandwich hybridization of dsDNA building blocks tailored with precise biotin residues, followed by the addition of streptavidin protein to the hybridization product. As shown in Figure 3A, two short DNA chains were designed as a precursor to facilitate the formation of long dsDNA building blocks. This hydrogel has been applied as a reservoir for alcohol oxidase (AOx) enzyme, resulting in its long activity and improved stability in the presence of various denaturants, such as freeze-thaw cycles, high temperature, and organic solvents. The soft nature of such hydrogels can potentially imitate living matrices and physiological environment, while their porous structure is a key benefit for accommodating cargo loading. Another type of hydrogels with DNA backbone is reported through electrostatic interaction between the positively charged polymers and negatively charged DNA chains.\textsuperscript{[66]} Moreover, nanomaterials, such as graphene, could form nonspecific interaction with short dsDNA and generate hydrogels with high mechanical properties just through a simple heating process.\textsuperscript{[69]} Photo-responsive hydrogels containing DNA backbone are also fabricated through the incorporation of azobenzene (AzO) moieties into DNA strands.\textsuperscript{[70]} This hydrogel exhibited sol–gel transition upon exposure to different wavelengths of light and was able to control the release of multiple drugs.

Contrary to the above-explained studies in which DNA was used as a backbone, DNA can also be served as a cross-linker to
Figure 2. A) Schematic illustration of the stepwise synthesis of the DNA hydrogel. Complementary primer 1 was used to produce elongated ssDNA1 products. Primer 2 and Primer 3 were used for chain amplification during MCA. Primer 2 was elongated to produce ssDNA2 that is complementary to ssDNA1. Primer 3 could create ssDNA3 that was complementary to ssDNA2. Therefore, ssDNA3 and ssDNA1 possessed exactly same sequences.

B1) To investigate the unusual properties of the DNA hydrogel, the gels with defined geometries of D, N, and A were prepared in moulds. B2) Water removal results in solid-to-liquid transition of the hydrogels, conforming the shape of the vial. B3) By reintroducing the water, liquid gels were returned to their original solid shapes within 15 sec. Hierarchical structure of the gels, shown in scanning electron microscopy image of (B3), is suggested as one of the parameters contributing to metaproperties of the gel. Reproduced with permission. [56] Copyright 2012, Nature Publishing Group. C) Synthesis procedure of DNA hydrogels via the hybridization of O1 with O2 and production of a dsDNA monomer, which can self-assemble. The dsDNA monomer O1-O3 comprises noncomplementary 15-base sequences, and therefore, cannot self-assemble. Reproduced with permission.[65] Copyright 2014, Wiley.

form hydrogels composed of other building blocks, such as protein and polymers, as the main backbone. [71,72] The enthusiasm toward using DNA as bridging molecule rather than the main building backbone lies on the limitations regarding large quantities of expensive synthetic DNA needed for the creation of programmable hydrogels with DNA backbone, as well as more feasibility of controlling gel reversibility and stability when DNA is used as a cross-linker. [16,73] In addition, tunability of thermal stability, stiffness, and viscoelasticity is achievable by changing the size and the number of branched DNA cross-linkers within hybrid hydrogels. [10,74–76] Polymers, polypeptides, and proteins are among the most studied backbones for the fabrication of DNA cross-linked hybrid hydrogels. [11,77,78] For instance, the synthesis of DNA cross-linked polypeptide backbones with precisely defined amino acid sequences and chain lengths imparts many unique features to the hydrogels, including excellent biocompatibility, biodegradability, and possibilities for versatile chemical modifications due to the many different functional groups present along the main chains of peptides. Programmable hydrogels of protein–DNA can also be prepared either by covalent chemical reactions or using physical entanglement. [79,80] A new creative method based on ssDNA hybridization is also reported, which allows orthogonal encoding of multiple proteins with easily programmable DNA sequences. [79] Due to the high stability, enzymatic degradability, and low toxicity, human serum albumin (HSA) was used as the backbone of hydrogel. HSA was cross-linked to multi-arm DNA without using any toxic catalyst, polymerization initiator, or organic solvent via sequential conjugation of polyethylene glycol (PEG) and ssDNA sticky ends to the polypeptide chains (Figure 3B). One of the main advantages of this system is very rapid gelation procedure, which might even allow in situ gelation at the target tissue by simply injecting both DNA and polypeptide copolymer components. PEG was also able to reduce immunogenicity and nonspecific protein absorption.
The stiffness was tunable in a broad range depending on the concentration of HSA-derived polypeptide copolymer and the 3-arm DNA cross-linker (DL1). The green fluorescent protein (GFP) and yellow fluorescent protein (YFP) were also used as model proteins for immobilization into the hydrogel.

Shape-memory hybrid hydrogels are also synthesized using DNA cross-linkers, representing an interesting property in which a programmed temporary shape can turn to the memorized original shape when placed in an appropriate environment or exposed to a trigger.\[^{[6,28,73,81]}\] Thermal, light, or magnetic stimuli are among the most studied triggers to activate different types of shape-memory hydrogels applied as sensors, materials for actuating microdevices, or controlled drug release.\[^{[6,28,49]}\] Therefore, recent attempts have been highly toward employing the information encoded in DNA sequences as versatile means for the synthesis of shape-memory hydrogels. In this context, a gel was formed using acrylamide chains modified with two types of pH-responsive DNA moieties, resulting in two types of cross-linkage within polymer chains.\[^{[28]}\] The first oligonucleotide structure consisted of thymine–adenine–thymine bridges, T-A·T, that forms at pH 7.0 and dissociate at pH 10.0. The second motif is a protonated cytosine–guanosine–cytosine unit, C-G·C\[^{+}\], formed at pH 5.0 and dissociated at pH 7.0. These two bridges provide the code for the generation of stimuli-triggered shape-memory DNA hydrogels in which, at pH 5 and 10, one of the cross-linking units provide the memory of the shaped hydrogel and the other...
cross-linking element provides the switching stimuli to trigger the transition of the system to shapeless quasi-liquid states. The whole procedure of the gel formation and its shape-memory behavior is shown in Figure 4. As shown in the SEM images, a porous cross-linked network was observed at pH 7.0 (panel I), which had lower pore size compared to the quasi-liquid systems generated at pH 10.0 and pH 5.0 (panels II and III). This observation could explain altered mechanical properties of the gel at various pHs. A similar shape-memory hybrid hydrogel was constructed using duplex DNA and pH-sensitive triplex DNA units as cross-linking units. It is worthy to point out that DNA sequences solely used for cross-linking of hybrid hydrogels result in highly swellable and less stable structures, unless substantial quantity of costly DNA cross-linker sequences is used. To circumvent this drawback, additional cross-linkers (e.g., responsive cross-linkers, such as disulfide bonds) can be incorporated in the structure of the gels to reinforce stronger structural integrity while retaining sequence-specific responses of the DNA cross-linkers.

4. Responsive DNA Hydrogels

The design of stimuli-responsive DNA-based hydrogels have attracted substantial attentions, mainly due to the structural property of nucleic acids, which allows the formation of a variety of dynamic assemblies with the capability of changing their macroscopic features by altering their conformation or integrity in response to various stimuli, such as temperature, small molecules (salts, ions, enzymes), and pH.

These hydrogels have been applied thus far for various purposes,
including switchable catalysis, sensors, controlled drug release, separation of substrates, catalyzed synthesis of conducting wires, tissue engineering, and the triggered activation of enzyme cascades.\textsuperscript{[68,70,79,85–88]} One of the main advantages of these hydrogels is the capability of loading different biomolecules into their matrix before the gelling process, leading to high efficient cargo encapsulation within the gels. For example, intercalation of doxorubicin (DOX) with DNA hydrogels and incorporation of a cytosine–phosphate–guanine (CpG) motif into the gel created a hydrogel with high immunological activity, responsiveness to serum nucleases, and synergistic effect to substantially inhibit a hydrogel with high immunological activity, responsiveness to the environment.\textsuperscript{[1,92]} When ionized, the matrix.

Taking this into account, Guo et al.\textsuperscript{[2,93]} have demonstrated the formation of DNA hydrogels that underwent reversible gel-to-solution transitions and can return to the original shape upon being triggered by different signals, such as ions. Shape-memory DNA hydrogels not only undergo stimuli-triggered phase transitions but also can possess physical or chemical memory code to recover into the original shape of the matrix.\textsuperscript{[2,93]} Taking this into account, Guo et al.\textsuperscript{[2,93]} have developed a pH-controlled shape-memory DNA hydrogel by copolymerization of acrylamide residues with acrydite modified with 1) cytosine-rich sequences (forming i-motif subunit) and 2) nucleic acids exhibiting self-complementarity. The cytosine-rich nucleic acid strands can self-assemble at pH 5.0 into an i-motif structure\textsuperscript{[1,94]} and disassemble to a random coil formation at pH 8.0 by dissociation of the i-motif structures, leading to the transition of the hydrogel to a “quasi-liquid” state. It was observed that when the produced DNA hydrogel was subjected to pH 8.0, it dissolved to an amorphous state (“quasi-liquid”) state, and after re-acidification to pH 5.0, the original structure was restored. The shape-memory ability lasted for only 4 h at 25 °C in the “quasi-liquid” state. Also, in this study, acrylamide chains copolymerized with acrydite units that were modified with both cytosine-rich sequences and self-complementary sequence could retain the memory ability in the “quasi-liquid” state for at least 40 h at 30 °C. The shape-memory feature of the hydrogel was attributed to the formation of cross-linking of i-motif and the DNA-bridging duplexes.

In another study, Yu et al.\textsuperscript{[4,95]} have also shown that shape-memory hydrogels based on bifunctional cross-linking units of duplex DNA and i-motifs made of acrylamide copolymer chains modified with acrydite nucleic acids containing cytosine-rich sequences, as subunits of i-motifs and self-complementary nucleic acids, can respond to two orthogonal triggers, pH and Ag⁺ ions. At pH 5.0 (state I), there was cross-linking interaction by the duplex and i-motif, resulting in the formation of the hydrogel. When neutralized to pH 7.4 (state II), the i-motif bridging units dissociated, leading to the quasi-liquid state. Furthermore, at pH 7.4, the hydrogel could recover its shape in the presence of Ag⁺ ions (state III), mainly due to the bridging of cytosine-rich tethers with C-Ag⁺-C bridged motif units, not being observed with other metal ions. Moreover, the removal of Ag⁺ (state IV) reformed the hydrogel to the shapeless quasi-liquid state that was able to restore its shape if re-acidified (state I). The visual observations were supported by micro-indentation experiments evaluating the Young’s moduli of the reversible signal-triggered states, showing high values for the bifunctional cross-linking of the hydrogel and smaller values for quasi-liquid state (state I: Y = 1640:265 Pa; state II: 341; state III: 1953:215 Pa; state IV: 344:5 Pa; state I: 1565:111 Pa). Scanning electron microscope (SEM) images showed very large pores in states II and IV (corresponding to low cross-linking) and small pores in states I and III (corresponding to high cross-linking levels). These hydrogels with the capability of responding to two orthogonal triggers are extremely relevant for controlled release of drugs from biomaterials.

Using triplex DNA structures based on Hoogsteen base interactions, it is possible to produce pH-responsive DNA hydrogels. Ren et al.\textsuperscript{[6,46]} have demonstrated the formation of DNA hydrogel at pH 7.4 by duplex Watson–Crick interactions through the mixing of two copolymer chains of acrydite-acrylamide, each one modified with a particular complementary domain. Decreasing the pH to 5.0 resulted in the separation of the duplex units and the protonated CG • C’ parallel domains favored the formation of triplex structure,\textsuperscript{[7,97,98]} which caused the separation of the hydrogel. The reversible transition between hydrogel and liquid phase was proven by macroscopic observation and by rheology characterization. Both G’ and G” values were very similar and between 1–2 Pa (liquid phase) at pH 5.0, whereas at pH 7.4, G’ was near 22 Pa and G” was 2 Pa. In the same study, another hydrogel was produced with the capability to undergo hydrogel–solution transitions under basic conditions, using TA•T parallel
domains, which stimulate triplex formations at neutral pH. Here, thymine-functionalized acrydite monomer was copolymerized with the acrylamide monomer, and the copolymer chain was mixed with adenine-rich nucleic acid at pH 7.0, yielding a triplex-bridged hydrogel. When increasing the pH to 10, the thymine residues were deprotonated, resulting in the separation of the triplex structures and the transition of the hydrogel to the liquid state. Furthermore, Coralyne, an anticancer drug that preferentially binds to TA$^*$ triplex structures,[8,99,100] was loaded in the cross-linked hydrogel and its release at pH 7.0 and pH 10.0 was analyzed. The loading of Coralyne could increase the stiffness of the hydrogel ($G' \approx 64$ Pa for loaded hydrogel and $G' \approx 48$ Pa for unloaded hydrogel). A slower release rate was observed at pH 7.0 (19% in 400 min) compared to pH 10.0 (56% in 400 min), attributed to the dissociation of the hydrogel at basic pH.

Recently, Li et al.[9,101] have successfully produced a stable supramolecular (G)-quadruplex hydrogel by self-assembly of guanosine (G), 2-formylboronic acid (2-FPBA), and tris(2-aminoethyl)amine (TAEA) in the presence of potassium chloride (KCl) in an aqueous solution for pH-triggered zero-order drug release. The produced DNA hydrogel possessed iminoboronate bonds between G, 2-FPBA, and TAEA, which allowed the connection of adjacent G-quartets, imparted pH- and glucose-responsive and viscoelasticity properties to the hydrogel ($G' > G''$).[10,102,103] X-ray powder diffraction (XRPD) and Fourier-transform infrared spectroscopy/nuclear magnetic resonance (FT-IR/NMR) could confirm the existence of G-quadruplex structures and existence of iminoboronate bonds, respectively. Also, SEM pictures showed a fibrous structure with cylindrical fibers ranging from 20 to 85 nm. Moreover, the hydrogel has shown a pH-dependent release profile of a model drug (methylene blue), with only 50% of the drug being release at pH 7.4 after 12 h compared to 80% release at pH 5.0 after the same time. The higher release at acidic pH was attributed to the dissolution of the quadruplex hydrogel by the breaking of the hydrogen bonds between guanosines and destruction of the imino bonds between 2-FPBA and TAEA due to protonation of the amino groups. Treatment of the hydrogel with higher concentrations of glucose also induced similar release profiles. Additionally, the hydrogel has shown a zero-order release kinetic, attributed to the dissolution of the gel at the surface without swelling of the polymer (confirmed by SEM, where no larger pores were observed after exposure to the stimuli). The authors suggested that this was a promising system for the controlled delivery of anti-inflammatory drugs in infection sites as well as anticancer drugs.

### 4.2. Molecular-Responsive DNA Hydrogels

DNA hydrogels can be synthesized to respond to different biomolecular cues in the environment, which is important for therapeutic and diagnostic purposes. Using this rational thinking, Chen et al.[11,104] have coated DOX-loaded metal–organic framework nanoparticles (NMOFs) with an ATP-responsive DNA hydrogel in order to control the release of the drug in the tumor site and enhance the efficacy of the anticancer therapy (Figure 5A). The DNA hydrogel was produced by hybridization chain reaction via the cross-linking of two DNA-functionalized polyacrylamide chains, forming a triplex-bridged hydrogel composed of anti-ATP (adenosine triphosphate) aptamer sequence in a caged configuration. The coating of the nanoparticles with the DNA hydrogel slightly increased the size of the particles (from $\approx 420$ to $\approx 680$ nm), enhanced the drug loading (both the model drug rhodamine 6G and DOX), and controlled its release. In fact, the modified particles prevented the leakage of the loaded

![Figure 5. Schematic illustration of the adenosine triphosphate (ATP) and glutathione (GSH)-responsive DNA hydrogels for targeting and controlled release. A) ATP-responsive DNA hydrogel coated metal–organic framework nanoparticles (NMOFs) loaded with DOX could efficiently target cancer cells and release the drug intracellularly due to the formation of responsive ATP-aptamer-ligand complexes. Reproduced under the terms of the Creative Commons Attribution 3.0 Unported Licence.[104] Copyright 2017, Royal Society of Chemistry. B) DNA hydrogels formed by two Y-shaped monomers, containing a targeting unit, and one linker. The particles could target cancer cells and release payloads intracellularly in the presence of GSH due to the cleavage of the disulfide bridges. Reproduced with permission.[105] Copyright 2015, American Chemical Society.](image-url)
Figure 6. A) Illustration of the mechanism of cloaking and release of tumor cells by the ATP-responsive DNA hydrogels. Anti-EpCAM aptamers bind to the surface of EpCAM expressing cells, triggering a hybridization chain reaction, leading to gelation and encapsulation of the cells. In contact with ATP, the gel denatures and releases the encapsulated cells. B) ESEM image of MCF-7 cells encapsulated in the DNA hydrogel. Scale bar is 20 μm. C) Encapsulation of AuNPs in the DNA hydrogel, due to gelation in the presence of cancer cells. D) Decloaking of the DNA hydrogel by ATP and consequent release of cells. Confocal images of live cells encapsulated in the hydrogel after release. Reproduced with permission [106]. Copyright 2017, American Chemical Society.

drug (only ≈8% released in the absence of ATP) and demonstrated a specific ATP-dependent drug release. When subjected to higher concentrations of ATP, higher release rates were observed. Moreover, there was no release when the particles were in contact with other nucleotide triphosphates (TTP, GTP, CTP), proving the specificity for ATP. The ATP-triggered release was attributed to the formation of ATP-aptamer complexes, leading to the separation of the caged ATP aptamer duplex nucleic acid and a lower degree of cross-linking, which diminished the stiffness of the hydrogel and increased its permeability to drug diffusion and release. The unloaded DNA hydrogel modified nanoparticles also showed good cytocompatibility in MCF-10A epithelial cells and MDA-MB-231 breast cancer cells, while cytotoxicity was favorably very high toward MDA-MB-231 for drug-loaded particles.

It can also be advantageous to design DNA-based drug delivery systems to release the payloads only inside the cells, for example, for gene therapy. In one study, DNA hydrogels capable of triggering the release of their payloads upon contact with the reducing agent, glutathione (GSH), present in the cells cytosol, were developed to selectively release therapeutic genes (Figure 5B).[12,105] The DNA hydrogel were formed by two Y-shaped monomers (A and B) and one linker, and their size was controllable by varying the ratio of the monomers. The A monomer was a building block assembled from three ssDNAs that each possessed one “sticky end” capable of hybridizing with the linker. The B monomer only had one “sticky end” and a strand consisting of an aptamer capable of recognizing specific cells for targeting purposes. Thereby, the B monomer could be considered as a building block to control the size of the hydrogels and to act as a targeting unit. The linker was a linear duplex DNA with two “sticky ends” complementary to the “sticky ends” of the monomers. Moreover, all the components possessed disulfide bridges which are stable in the absence of GSH but cleaves in its presence, rendering stimuli-responsive properties to the DNA hydrogel. When mixed together, spherical hydrogels with the size of 144 nm were formed by self-assembly. To confirm response of the hydrogel to GSH, gels were incubated in the presence or absence of GSH and analyzed by agarose gel. Only after treatment with GSH, DNA fractions appeared in the agarose gel, which indicates the disulfide linkages are being cleaved by GSH. Furthermore, by adding a targeting aptamer for A549 cells and incorporating therapeutic genes, the authors showed that not only is the system capable of specifically targeting cells but also could deliver genes capable of modulating protein expression and inhibit cell proliferation and migration. Altogether they have shown the ability of the produced DNA hydrogels for targeted and stimuli-responsive gene regulation therapies.

Another interesting use of molecular-responsive DNA hydrogels was proposed by Song et al.[13,106] In this study, a DNA hydrogel capable of signal-triggered in situ gelation and also denaturation was developed to specifically capture circulating tumor cells (CTCs) for cancer diagnosis (Figure 6A). For this purpose, aptamer-initiator biblocks were produced using anti-EpCAM aptamer to anchor the DNA specifically to the surface of cancer cells, as these cells are known to overexpress this receptor. The specificity of the binding was proven by observing colocalization on two-color confocal microscopy, after incubating fluorophore labelled aptamer-initiator biblocks with membrane labelled EpCAM-positive MCF-7 cells. No fluorescence signal of the aptamer was detectable when incubated with EpCAM-negative HEK-293 cells. When the aptamer-initiator biblocks bonded to
the surface of the MCF-7 cells, a clamped hybridization chain reaction was triggered, leading to the gelation of the DNA and consequent encapsulation of the cells (Figure 6B). The hydrogel formation was visualized by adding gold nanoparticles (AuNPs) to the cell solution. Herein, upon contact of the aptamer with the cells and consequent gelation, AuNPs were encapsulated and the color of hydrogel turned to red (due to plasmon resonance adsorption of the AuNPs, while the buffer became a transparent solution (Figure 6C). Moreover, the developed system could also specifically detect and encapsulate MCF-7 cells present in blood. Furthermore, an ATP-aptamer was incorporated into the clamped hybridization chain reaction, which in the presence of ATP, changed its conformation from unfolded to tertiary state that subsequently forced the collapse of the hydrogel and release of the captured cells (Figure 6D). The captured cells maintained their viability, morphology, and molecular properties while encapsulated and also after being released from the gels (Figure 6D), posed as a promising system for capturing or delivery of live cells.

4.3. Enzymatic-Responsive DNA Hydrogels

Enzymes, due to their ability to cut DNA chains, can be used to influence the sol–gel state of DNA hydrogels by changing their conformation. For example, restriction enzymes (restriction endonucleases), can cut dsDNA at very specific recognition sites, known as restriction sites. Using this concept, Xing et al. have developed a self-assembled DNA hydrogel made of a Y-shaped structures composed of three ssDNA strands with three segments each (one “sticky end” to connect with the linker and two segments to hybridize with the other strands) and a linear duplex linker composed of two ssDNAs with two “sticky ends”, in which a restriction site was inserted. In order to test the specificity and responsiveness of the developed hydrogel to enzymes, two hydrogels were produced using two different restriction sites specific for BamHI and EcoRI enzymes and both hydrogels were incubated with both enzymes. The gels have only suffered gelation (specific for BamHI and EcoRI enzymes) and both hydrogels were produced using two different restriction sites.

Enzymes, due to their ability to cut DNA chains, can be used to influence the sol–gel state of DNA hydrogels by changing their conformation. For example, restriction enzymes (restriction endonucleases), can cut dsDNA at very specific recognition sites, known as restriction sites. Using this concept, Xing et al. have developed a self-assembled DNA hydrogel made of a Y-shaped structures composed of three ssDNA strands with three segments each (one “sticky end” to connect with the linker and two segments to hybridize with the other strands) and a linear duplex linker composed of two ssDNAs with two “sticky ends”, in which a restriction site was inserted. In order to test the specificity and responsiveness of the developed hydrogel to enzymes, two hydrogels were produced using two different restriction sites specific for BamHI and EcoRI enzymes and both hydrogels were incubated with both enzymes. The gels have only suffered gelation (specific for BamHI and EcoRI enzymes) and both hydrogels were produced using two different restriction sites.

Therapeutic efficacy of the self-assembled Y-shaped DNA hydrogels was assessed by changing the conformation of the hydrogel by addition of specific enzymes. The developed system was tested using two different enzymes (Restriction enzymes), which were able to digest DNA, leaving only the CB[8] hydrogel network intact. Similarly, when in contact with cellulase, the CB[8] network is specifically destroyed and the DNA hydrogel remains intact. Furthermore, when both enzymes were applied at the same time, the system fully degraded. This was concluded through visual observation (Figure 7B) and analysis of rheological studies (Figure 7C). The improvement of the mechanical characteristics and the ability to control the degradation of the system using two different enzymes makes it a promising system for biomedical applications, such as drug delivery.

Another enzymatic-responsive DNA-based hydrogel was produced by Gacanin et al. for therapeutic effect against osteoporosis by controlling the release of C3 toxin, which inhibits actin-dependent processes on osteoclasts and reduces their formation and resorption. For this purpose, a hydrogel was produced by interaction between a PEG and ssDNA modified chHSA polyolpeptide copolymer with multiam DNA cross-linker containing one arm to increase the cross-linking degree and the other to load up to two molecules of recombinant C3 toxin (C2IN-C3lim). In order to conjugate C2IN-C3lim to the DNA cross-linker, a mutant of this protein was created by replacing glycine at position 205 with cysteine, forming C2IN-C3lim-G205C, capable of accomplishing thiol-based bioconjugation. Importantly, C2IN-C3lim-G205C maintained its activity after conjugation to the cross-linker and sustained release was achieved through hydrogel degradation in the presence of DNase I. Moreover, the hydrogel was proven to be biocompatible, and when stabilized with chitosan, its stability increased to over 30 days as compared to 1 day for samples without chitosan. This hydrogel had also self-healing behavior and could be injected through a needle.

Aiming to develop a multifunctional nanosystem for cancer therapy, Zhang et al. developed quantum dot (QD) DNA hydrogels to load different molecules (DOX and siRNA), target cancer cells, and specifically control the release of therapeutics owing to the action of DNase I inside the cells. Firstly, DNA-templated QDs were produced by a phosphorothioate domain which binds to the surface of the QD, a spacer, and a DNA-binding domain with phosphate linkages. The formed QDs were mixed with a DNA three-way junction, and self-assembled to a gel through the hybridization of the phosphate domain from the QD with the complementary target DNA. Furthermore, the DNA hydrogel was covered with a ZnS shell to improve its biocompatibility. The formation of the hydrogel was confirmed by rheological studies, showing G’ > G”, and SEM images in which it was possible to observe a porous cross-linked structure. Moreover, the size and degree of swelling of the produced hydrogels could be controlled. The modification of the surface of the hydrogel with a DNA-based aptamer specific to a cell surface receptor significantly enhanced the cellular uptake. Importantly, the hydrogel was able to encapsulate DOX and its release was shown to be DNase I dependent since no DOX release was observed after 72 h of incubation in PBS at 37 °C. However, when DNase I was present in the release media, DOX was released. Furthermore, the drug-loaded hydrogels have been shown to be significantly more effective on killing cancer cells, which was attributed to the ability of the controlled release of the drug. This translated in an enhanced therapeutic effect in cancerous mice model, as the drug-loaded...
A) Schematic illustration of the formation and degradation of the double network enzyme-responsive hydrogel through supramolecular interactions. Cucurbit[8]uril (CB[8]) reacts through host–guest interaction with carboxymethyl cellulose (CMC-phe) forming a hydrogel and the DNA Y-scaffold hybridizes with DNA linker forming a DNA hydrogel. When mixed together, a double network hydrogel was produced, with combined mechanical properties of each single hydrogel. B) Enzymatic response of the double network hydrogel. EcoR I digests the DNA hydrogel, leaving the CB[8] gel intact, while cellulase degrades the CB[8] gel leaving the DNA hydrogel intact. Both enzymes together are able to completely degrade the double network hydrogel. C) The mechanical properties of the double network hydrogel were characterized before and after digestion with the enzymes. The changes on $G'$ and $G''$ are in agreement with the visual observations. Reproduced with permission. [108] Copyright 2015, Wiley.

In another study, photo-responsive DNA hydrogels were prepared by cross-linking DNA with ethylene glycol diglycidyl ether (EDGE), which binds to the guanine bases of the DNA molecules,[20,111] and plays photosensitive role.[21,112] Herein, when the produced hydrogel was exposed to UV light, EDGE suffered degradation leading to lower degree of cross-linking and consequent swelling of the hydrogel, increasing the gel mesh size and inducing the release of DNA and different molecular weight hydrophilic solutes (BSA, FITC-dextran). Moreover, the degradation of the hydrogel and release of DNA was dependent on both the radiation wavelength and the concentration of the EDGE cross-linker. Lower energy radiation (380–400 nm) and lower concentrations of EDGE (less cross-linked gels) induced higher release rates. Using EDGE as cross-linker, Costa et al.[22,113] have synthesized new biocompatible photo-responsive polyamine pDNA nanogels. The developed DNA nanogels were produced through plasmid (pVAX1-LacZ and pcDNA3-FLAG-p53) condensation with different polyamines, such as spermine, protamine sulfate, and polyethylenimine (PEI), followed by cross-linking with EDGE. The produced photosensitive DNA nanogels were either spherical or ellipsoidal with sizes ranging from 80 to 400 nm. The size of nanogels was found to be dependent on the concentrations of EDGE (higher concentrations caused higher cross-linking degrees and led to smaller sizes). The DNA nanogels showed photo-responsive properties due to the presence of EDGE. Upon exposure to UV light (400nm), EDGE degraded and consequently led to lower cross-linking degree of the gel and controlled release of pcDNA3-FLAG-p53 gene and...
different loaded anticancer molecules (DOX, epirubicin, and paclitaxel). In general, high biocompatibility combined with stimuli-responsive co-delivery of gene and anticancer drugs is found promising for further investigation of DNA-based nanogels in multitherapy.

4.5. Temperature-Responsive DNA Hydrogels

When heated above a certain temperature (80–90 °C), DNA denatures due to the breakage of hydrogen bonds between the double helix and forms two separated ssDNA with random coil conformation. The denaturation may be reversible by slow cooling down of the DNA solution. Herein, it is possible to take advantage of these properties to develop thermo-responsive DNA hydrogels for controlled release purposes. By combining AuNPs with DNA hydrogels, Song et al.\textsuperscript{23,114} could develop a thermosensitive drug delivery system for controlled release of DOX. In this study, DNA hydrogel was produced by enzymatic assembly of X-shaped DNA composed of three arms with “sticky sites” and one disabled arm, which controlled the cross-linking and consequently the size of the hydrogel. DOX was intercalated into the DNA hydrogel and positive charged AuNPs were then incorporated into the drug-loaded DNA hydrogel by self-assembly through electrostatic interactions. The introduced AuNPs, upon light irradiation (660 nm), were excited at their plasmon resonance and generated heat shock that led to disassembly of the hydrogel and subsequent release of the drug and AuNPs (Figure 9). Furthermore, the system was biocompatible and only upon light irradiation, it induced toxicity on B16F10 mouse melanoma cells, both in vitro and

Figure 8. A) Schematic illustration of photo-responsive properties of DNA hydrogels. The incorporation of azobenzene (Azo) in trans-state into the backbone of the DNA-polymer conjugates allows hybridization and formation of a hydrogel. Transition gel–sol is reversible and light dependent, as when upon UV light, Azo isomerizes to the cis-state, leads to the solution state and consequent release of the payloads. B) Controlled release of DOX by UV–Vis irradiation. C) Cytotoxicity of the DOX-loaded hydrogels after incidence of UV and visible light. Reproduced with permission.\textsuperscript{70} Copyright 2011, American Chemical Society.
in vivo. The observed cytotoxicity was then attributed to the controlled release of DOX plus increment of temperature on the tumor, which is known to induce tumor ablation.\cite{24,115,116} In another study, Yata et al.\cite{25,117} used AuNPs and Au nanorods (AuNRs) to produce thermo-responsive DNA hydrogels for photothermal cancer immunotherapy. AuNPs and AuNRs were firstly modified with oligodinucleotides containing CpG sequences and then mixed with hexapodnas, forming double strands and a hydrogel. Upon laser irradiation (532 nm), the temperature of the gels increased, leading to the disruption of the hydrogel and release of both DNA (CpG motifs) and AuNPs. To study the immunostimulatory properties of the hydrogel, both hydrogels (subjected or not to laser irradiation) and the released materials were incubated with RAW264.7 macrophages and mouse dendritic TLR9-positive DC2.4 cells after irradiation and secretion of TNF-$\alpha$ and IL-6 were analyzed. In the presence of laser irradiation, the hydrogel and the released compounds significantly increased the secretion of both cytokines, while in the absence of irradiation, the cells were hardly activated. Moreover, after injecting the hydrogels into mice bearing EG7-OVA tumors and subjecting them to laser irradiation (780 nm), the temperature of the hydrogels and tumors increased, and the tumor growth was significantly inhibited compared to nonirradiated mice.

4.6. Magnetic-Responsive DNA Hydrogels

Ma et al.\cite{12,26} have introduced magnetic nanoparticles into a DNA hydrogel in order to induce magnetic-responsive properties to the gel. First, magnetic nanoparticles (MNs) were modified with an ssDNA segment, forming DNA modified MNs (DMN). Afterward, DMNs were mixed with a DNA Y-scaffold and ds-DNA linkers to produce a hydrogel, in which the MNs were well dispersed. Rheological tests showed that $G' > G''$, confirming the gel-like state, and that the introduction of the nanoparticles did not change the mechanical properties of the DNA hydrogel. Upon subjected to a magnetic field, the DNA hydrogel distorts its shape from spherical to ellipsoidal and could be moved. Moreover, it could transition between gel-sol state using temperature (40 °C) and enzymes (endonuclease).

5. Biomedical Applications of DNA Hydrogels

5.1. Therapeutic DNA Hydrogels

Exploring biomedical applications of DNA hydrogels has been of particular interest in recent years.\cite{1,90} For instance, DNA hydrogels are used as ideal platforms for controlled drug delivery, due to the highly efficient cross-linking maintenance of DNA hydrogels in physiological environment, which allows in situ encapsulation and preservation of payloads.\cite{20} Porcine insulin and camptothecin have been successfully co-loaded within a DNA hydrogel in situ with an encapsulation efficiencies of nearly 100%.\cite{29} Drug release was sustained without any initial burst release. Manipulation of the degradation rate and release profile from the hydrogel was also feasible through the adjustment of the initial concentration and type of branched DNA. Nonspecific electrostatic interaction between positively charged drug molecules and negatively charged DNA is a common approach for drug loading into DNA hydrogels.\cite{89}
For example, a DNA physical meta-hydrogel is developed to control drug release profiles of DOX and insulin loaded through ionic interaction with opposite charges present in the hydrogel network.[56] Cancer chemoimmunotherapy is also reported via drug-loaded cytosine-phosphate-guanine (CpG)–DNA hydrogels.[89] CpG sequence containing DNA hydrogels are studied due to their ability to elicit immune responses.[20,118,119] For example, DNA hydrogel containing unmethylated CpG dinucleotides was used to stimulate innate immunity through Toll-like receptor 9 and act as adjuvant for increasing the immune responses of ovalbumin (OVA) antigen incorporated into the gel.[20] In this study, higher adverse reactions of OVA injected with complete Freund's adjuvant or alum was observed compared to OVA-loaded CpG-DNA hydrogel.

In a novel study, injectable hybrid hydrogels were prepared using ssDNA and drug-specific polyaptamer sequences combined with graphene oxide (GO) nanosheet as a physical crosslinker.[120] Single-step RCA was used for a DNA template that was designed to contain 1) kanamycin (Kan)-aptamer sequence for efficient and specific drug loading and 2) a GO-binding 12-mer oligo A sequence (Figure 10A). It was shown that oligo T sequences of RCA products could facilitate networking interaction of the DNA and GO to form hydrogels. The polyaptamer-GO (PA-GO) hydrogel showed a swelling ratio of 65% within 2 h, as well as a viscoelastic property suitable for injection. Kan was the model drug that could specifically bind to PA-GO hybrid hydrogels with a loading efficiency of 58% compared to 1.5% for gentamicin. This was mainly due to the specific aptamers for Kan in the structure of the hydrogel. Kan-loaded PA-GO hydrogels exerted antibacterial activity against both Gram-negative and Gram-positive bacteria. DNA-carbon dot (CD) hybrid hydrogel are also constructed for sustained drug release.[85] In the presence of DOX, phosphoramidate linkage could conjugate amine-functionalized CDs to 5'-phosphate termini of Cytosine (C) rich ssDNA to produce drug-loaded hydrogels (Figure 10B). Sol–gel transition of CD-DNA hybrid hydrogel was detectable by
changing the pH of the medium from alkaline to neutral. Higher rate of drug release and dissolution of the hydrogel was achieved in acidic pH that is relevant to tumor microenvironment. The pH-dependent drug release is attributed to the lower electrostatic interaction between CDs and DOX and more buffer diffusion into the gel.[121] Moreover, intermolecular i-motif structure of CD–DNA disrupts in acidic pH and contributes to faster drug release.

Effective multi-therapy can also be achieved by DNA hydrogels. Co-loading of AuNRs and DOX into DNA hydrogels is performed for dual thermo- and chemotherapy.[19] Electrostatic interaction and DNA-binding interactions caused incorporation of AuNRs and the drug into the gel, respectively. The procedure of nanoporous gel formation is depicted in Figure 10C. Strong binding affinity of the payload molecules could avoid uncontrolled drug release, while NIR triggering of the gel resulted in thermal denaturation of the DNA hydrogel and drug release. Dual thermo- and chemotherapy was very efficient for tumor ablation in animal models.

In addition to the bulk 3D DNA hydrogels, micro- and nanosized hydrogels are developed for drug delivery purposes.[110,122,123] A novel method is presented by Liao et al.[123] to construct DNA–acrylamide-based hydrogel microcapsules (Figure 11). CaCO₃ microcapsules were loaded with tetramethylrhodamine modified dextran (TMR-D) model drugs and then alternatively coated with positively charged poly(allylamine hydrochloride) (PAH) and a layer of negatively charged polyacrylic acid (PAA). PAA was covalently linked to an amine modified nucleic acid (1) promoter needed for the assembly of the microcapsule shells. Acrylamide copolymer chains Pₐ and Pₐ were modified with the DNA hairpin Hₐ (2) and the hairpin conjugate Hₐ (3)/(4), respectively. Pₐ and Pₐ were responsible to create ATP-responsive hydrogel microcapsules. Anti-ATP aptamer sequence was available in hairpin Hₐ of polymer chain Pₐ in a caged configuration (marked in red). The promoter (1) could open hairpin Hₐ (2) and yielded a toehold tether, which subsequently opened hairpin Hₐ, formed a duplex between (2) and (4), and finally led to the cross-linking of Pₐ and Pₐ. Besides, the opening of hairpin Hₐ resulted in the creation of a single-stranded domain that opened hairpin Hₐ and assisted the hybridization chain reaction, which led to the cross-opening of the hairpins and the synthesis of a cross-linked acrylamide shell composed of Pₐ and Pₐ chains bridged by the (2)/(3)/(4) units. EDTA was then used to dissolve the CaCO₃ core and obtain microcapsules loaded with TMR-D. The exposure of the microcapsules with ATP could dissociate the duplexes of (2)/(4) and partially separate the shells via the formation of ATP-aptamer complexes, resulting in the diffusional release of the cargos (Figure 11A). SEM images of the particles in each step and release profile of TMR-D from the ATP-responsive hydrogel microcapsules are shown in Figure 11B,C. The same concept is recently used for the coating of metal–organic framework nanoparticles with stimuli-responsive nucleic acid-polyacrylamide surface layers for controlled drug release.[124] Using a creative and novel design, 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) liposomes served as both noncovalent cross-linkers and nanocarriers for hydrogel formation and responsive drug delivery.[125] Initially, free radical polymerization was performed to copolymerize acrydite modified ssDNA with acrylamide. The obtained product was then hybridized with the cholesteryl–tetraethyleneglycol (TEG) modified complementary DNA stand. Hydrogel was formed upon the mixing of the functionalized polyacrylamide copolymer with liposomes due to the hydrophobic interaction between cholesteryl groups and the lipid bilayer of liposomes. Small hydrophobic and hydrophilic model drugs were separately entrapped to liposomes for controlled drug release. To achieve the enzymatic responsiveness, a specific recognition site (5′-GAATTC-3′) of restriction endonucleases, ECoR I,
was encoded into the DNA sequence. The thermal-responsive property of the gel was also attributed to the intrinsic behavior of DNA.

5.2. DNA Hydrogels for Biosensing

Recently, DNA has been applied to manufacture biosensors, which are powerful tools in monitoring biological or biochemical processes in medicine. Target stimuli-responsive DNA hydrogels are considered as sensitive, portable, selective, and cost-effective sensing platforms.[126,127] In a typical DNA hydrogel biosensor, payloads should be first caged within the DNA hydrogel comprised of a synthetic polymer as a backbone and a functional DNA (such as DNAzyme and aptamer) as a cross-linker.[128] Exposure of the gel to the target molecules will trigger the gel-to-liquid phase transition of the hydrogel, cargo release, and subsequently the generation of a signal for sensitive detection. A plethora of reports have used this strategy for the detection of a wide range of targets, including ions, live cells, proteins, viruses, or bacteria.[126–133] A series of DNA aptamers can cross-link to linear polymers, such as polyacrylamide chains through their high binding affinity to various targets in order to form target-responsive DNA hydrogels for the detection of biological molecules.[17,134] Polymer free DNA hydrogel sensors are also developed by means of DNA ligase or DNA hybridization.[135] For example, an aptamer-functionalized DNA hydrogel was prepared by Zhang and coworkers for thrombin detection.[136] AuNPs and QDs were incorporated into the gel as signal indicators. The DNA hydrogel was composed of Y-shaped DNA and linker DNA (L-DNA), containing 1) an aptamer sequence with two different recognition sites (APT15 and APT 29) for thrombin, and 2) the complementary sequence. AuNPs were encapsulated within the gel. Adding a thrombin free buffer could form a colorless solution on the top layer of the solution and a red layer of AuNP containing hydrogel in the bottom of tubes. Addition of the thrombin to the tubes led to the gel dissolution and the release of the AuNPs to the upper colorless solution due to the competitive binding with the L-DNA aptamer. In another study, AuNPs were incorporated into the DNA hydrogel to retain their catalytic capability in serum for the detection of glucose and hydrogen peroxide.[87] As shown in Figure 12A, a circular DNA template was hybridized using primer-1 at 25 °C for 2 h. The hybridized product was then incubated with Phi29 DNA polymerase at 30 °C for 4 h to launch RCA. The RCA product was then mixed with primer-2 and primer-3 and incubated at 30 °C for 20 h to launch MCA. The final product was treated at 65 °C for 10 min to terminate the reaction and obtain a precursor, which converts to a hydrogel after cooling down the solution and centrifugation. To produce AuNPs@DNA hydrogel, AuNPs were added to the DNA hydrogel precursor and incubated at 90 °C for 30 min before cooling down to 25 °C and centrifugation. For glucose sensing, glucose oxidase (GOx) could be easily incorporated into the hydrogel through the incubation of GOx with AuNPs@DNA hydrogel under agitation (4 °C, 1 h). Visual appearance of the gel, SEM image of AuNP-loaded gel, and schematic illustration of sensing mechanism is shown in Figure 12B–D.

5.3. DNA Hydrogels as Template for the Synthesis of Nanoparticles

A number of DNA-based hydrogel materials have been investigated as template for the synthesis of nanoparticles.[137–141]
Generally, the strong affinity of transition metals to nucleic acids has provided an opportunity for DNA to work as template for the synthesis of metal-based nanomaterials, as well as metallization of folded DNA condensates and self-assembled “DNA origami.” All these metallization approaches are limited to 1D or 2D DNA templates. Therefore, expanding the construction of the templates to 3D molecular architectures is immensely attracting plenty of attention in order to create a bulk 3D structure with uniform distribution of nanometals that can be used for different purposes, such as sensors and diagnostics. Concentrating of transition metal ions by DNA stems from the presence of purine and pyrimidine bases as well as phosphate groups in the backbone of DNA, which can act as efficient chelation sites. This is the main logic behind the extraction of transition metals from aqueous solutions by DNA hydrogel as an absorbent. This finding has prompted scientists to use DNA hydrogels for controllable synthesis of metal nanoparticles. For example, as shown in Figure 13, the synthesis of AuNPs inside matrix of Au(III)-absorbed hydrogel is reported through direct reduction, applied as environmentally friendly and sustainable hybrid for catalytic activities. Similar approach is also presented for the stabilization of photochemically synthesized silver nanoparticles by nontoxic hydrogels composed of positively charged protonated 2',4',6'-tris(4-pyridyl)pyridine supramolecular network and calf thymus DNA, which is negatively charged due to the phosphate backbone. This platform allowed efficient stabilization of Ag NPs photochemically synthesized under the direct exposure of sunlight.

6. Conclusion and Outlook

The application of DNA has been expanded far beyond its role in genetics. DNA hydrogels are found interesting in a variety of clinical purposes both as nanosized and bulk 3D networks. Here, we have reviewed the major advances in DNA self-assembly to develop functional materials for biomedical applications. DNA is a key polymer for controlling the function of other materials when a hybrid hydrogel is prepared. DNA-based macromaterials can be prepared with only DNA, DNA as backbone, or DNA as cross-linker. DNA can also serve as responsive materials in which the properties of the macroscopically visualized gel depend on nanoscale internal structures of DNA molecules and other motifs of the gel.

There has been a plethora of DNA-based structures developed in the last decade with the aim of implantation, biosensing, controlling drug release rate, and targeting specific cells in the body. Nevertheless, many issues remain to be addressed, including, pharmacokinetic profile and degradation of the DNA-based materials within the body, long-term safety, as well as finding new areas of applications, such as 3D printing as well as shear-thinning injectable DNA gels for cell therapy and tissue engineering. The later application is mainly due to the hydrated nature of DNA hydrogels, which can mimic the properties of the natural tissues and micro-environment of cells, fueling the rapid interest for the design of DNA-based tissue engineering scaffolds. In addition, the ease with which the mechanical properties of DNA cross-linked gels can be changed suggests that they would be useful for
cell growth and tissue repair. By selecting an appropriate gelation mechanism, cells can be encapsulated in DNA hydrogels without significantly altering their viability or activity.

Further attempts are also needed to develop new innovative matrices of DNA hydrogels with more favorable viscoelastic properties and swelling performance, which can enhance the biological interaction of the gels with the host body while reducing foreign body reactions. There are also a number of considerations for designing practically applicable DNA-based materials in medicine, including precise DNA sequence design, interactions between DNA and other molecules within the gel structure, and introduction of the stimuli in the materials. Furthermore, improving the stability of DNA self-assemblies needs further attention since it is currently a remarkable challenge to resist DNA to harsh environments or organic solvents, limiting the synthesis method of hybrid DNA hydrogels. Therefore, more researches are imperative to understand what is the impact of each component of the gel on its physicochemical and biological properties. Last but not the least, it is worthy to point out that there is much room for expanding the biomedical applications of DNA in future through reducing the cost of synthetic DNA, which is awaiting further development in other areas of science, including synthetic chemistry and molecular biology.

Acknowledgements

M.-A.S. acknowledges Iran’s National Elites Foundation and Iran Nanotechnology Initiative Council for financial supports. T.B.-R. acknowledges financial support from the Fundação para a Ciência e a Tecnologia (grant no. SFRH/BD/110859/2015). H.A.S. acknowledges financial support from the University of Helsinki Research Funds, the Sigrid Juselius Foundation (Decision no. 4704580), the Helsinki Institute of Life Science, and the European Research Council under the European Union’s Seventh Framework Programme (FP/2007–2013, Grant no. 310892).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

biosensors, DNA assemblies, DNA hydrogels, drug delivery, hybrid hydrogels, responsive

Received: April 8, 2018
Revised: May 4, 2018
Published online: June 21, 2018
