Biomedical Applications of DNA-Based Hydrogels

Jasmina Gačanin, Christopher V. Synatschke,* and Tanja Weil*

Nucleic acids are gaining significant attention as versatile building blocks for the next generation of soft materials. Due to significant advances in the chemical synthesis and biotechnological production, DNA becomes more widely available enabling its usage as bulk material in various applications. This has prompted researchers to actively explore the unique features offered by DNA-containing materials like hydrogels. In this review article, recent developments in the field of hydrogels that feature DNA as a component either in the construction of the material or as functional unit within the construct and their biomedical applications are discussed in detail. First, different synthetic approaches for obtaining DNA hydrogels are summarized, which allows classification of DNA materials according to their structure. Then, new concepts, properties, and applications are highlighted such as DNA-based biosensor devices, drug delivery platforms, and cell scaffolds. With the 2018 Nobel Prize in Physiology or Medicine being awarded to cancer immunotherapy underscoring the importance of this therapy, DNA hydrogel systems designed to modulate the immune system are introduced. This review aims to give the reader a timely overview of the most important and recent developments in this emerging class of therapeutically useful materials of DNA-based hydrogels.

1. Introduction

The great progress in DNA synthesis and the consequent accessibility of larger DNA quantities has prompted a material revolution by implementing DNA as a functional entity in the design of biohybrid materials.[1] Modern DNA synthesis techniques enable rapid preparation or amplification of effectively any DNA sequence in large quantities by automated techniques, polymerase chain reaction (PCR), or production in microorganisms.[2,3] In comparison to other biopolymers, such as proteins or polysaccharides, DNA consists only of a small set of nucleic

acid monomers. However, their highly specific and predictive interaction between the monomer units with the respective complementary nucleic acids (AT and GC) facilitates spontaneous and convenient programming of synthetic 2D and 3D structures in solution.[4] Even small DNA building blocks like DNA bricks can be combined to produce predictable assemblies of thousands of individual components.[5] Furthermore, DNA has great mechanical rigidity, thus it can serve as a rigid rod spacer for the precise positioning of tethered nanoparticles or functional molecular components along the polymer chain.[6,7] Recently, the development of more complex DNA scaffolds allowed the spatial arrangement of functional proteins within multifunctional assemblies.[8] Moreover, DNA-based materials can exhibit dynamic and adaptable properties due to the supramolecular nature of the interaction of complementary nucleic acid sequences. These materials can, e.g., present bioactive molecules in a dynamic and reversible fashion, which allows controlling their cellular interactions.[9]

Moreover, the high physicochemical stability of DNA nanostructures[10] can be modulated by external triggers. While DNA double strands show a distinct thermally triggered dissociation behavior (melting temperature), an increase in thermal stability of DNA double helices can be achieved through intercalating agents, like psoralen, or via photo-cross-linking.[11–13] Finally, a large number of tools are provided by nature in the form of highly specific enzymes, such as endonucleases,[14–16] exonucleases,[16,17] polymerases,[16–19] and ligases[16,17] that allow the DNA material to be processed or degraded efficiently and with a high level of precision.

Based on these features, DNA has stimulated new directions in materials design (**Figure 1**). For instance, its precise hybridization into 2D and 3D objects has culminated in the development of several bottom-up fabrication processes such as DNA origami, where a circular, single-stranded DNA template is predictably folded into precise nanostructures with short DNA sequences, called staple strands.[20] Furthermore, as DNA objects are now becoming more readily available, they become increasingly useful, e.g., as precision templates for the construction of precise 2D and 3D polymeric architectures.[21–23] Since DNA is a strongly hydrophilic polyelectrolyte, it can absorb large amounts of water, sparking an interest in DNA for hydrogel design.[24,25] In this respect, DNA is an excellent component for the construction of supramolecular hydrogels, as it imparts important features such as biocompatibility, controlled biodegradability,

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permeability for nutrients, adjustable mechanical properties, stability against proteases, the ability for self-healing, and responsiveness to various stimuli to the hydrogel.[26,27] In comparison to DNA, many other noncovalently cross-linked hydrogels derived from natural sources like alginate or gelatin, lack this level of versatility and structural programmability.[26] Therefore, smart DNA hydrogels combine many desirable features for preparing customized materials for biomedical applications that cannot easily be achieved with other materials.

In this review, we summarize the underlying design principles for the construction of DNA-based hydrogels and highlight their applications in different fields of biomedicine (**Figure 2**). In contrast to other recent reviews on DNA hydrogels,[28–32] we particularly focus on their applications in emerging fields such as biosensing, drug delivery, immunotherapy, and tissue engineering and we discuss their unique features as well as current limitations.

2. Design of Nucleic Acid-Based Hydrogels

DNA-based hydrogels have been prepared based on different design principles.[28–31,33] The selected design and preparation methods have a strong impact on the characteristic features of DNA hydrogels and thus determine their respective biomedical applications. In this section, we introduce 1) hydrogels that consist entirely of DNA, and 2) the usage of DNA as versatile and biocompatible cross-linkers in combination with synthetic polymers as well as 3) materials containing short DNA sequences as functional grafts providing access to customizable hydrogels with unique bio-specificity.

2.1. "All-DNA" Hydrogels based on Polynucleotide Scaffolds

Today, based on the seminal work of Luo and colleagues, who achieved the first self-supporting all-DNA hydrogel, DNA can be considered as a building unit for bulk macroscale materials. This hydrogel reported by Um et al. in 2006 was prepared from branched and flexible 3D DNA, which was designed to associate with other complementary strands under network formation.[34] Several strategies to produce hydrogels made entirely from DNA sequences followed and have since been reported.[13,35–37] In the first approach, preformed DNA building blocks are mixed in solution, which then form a 3D network due to the interactions of complementary DNA strands on separate building blocks (**Figure 3**a). Binding between the building blocks can be achieved through hybridization of DNA sticky ends,[35] i-motifs,[36] or enzymatic ligation[34] as depicted in Figure 3a,b. The i-motif represents a tetrameric structure of cytosine-rich DNA sequences that self-assemble into a four-stranded complex.[36,38] These assemblies are formed at pH 5 and dissociate at pH 8, thus introducing a rapid, pH-responsive sol–gel transition of i-motif-containing materials.

All-DNA gels were also generated in more recent work by in situ formation of the building blocks, e.g., by enzymatic polymerase amplification reactions. Using branched, Y-shaped primers that are thermally stabilized by psoralen treatment, hydrogels can be prepared by PCR via extending and connecting the forward and reverse primers.[13] An intriguing example of

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gel formation by DNA polymerization uses combined rolling circle amplification and multi-primed chain amplification to build extremely long DNA sequences that eventually form gels through entanglement rather than predetermined cross-linking points (Figure 3c).[37]

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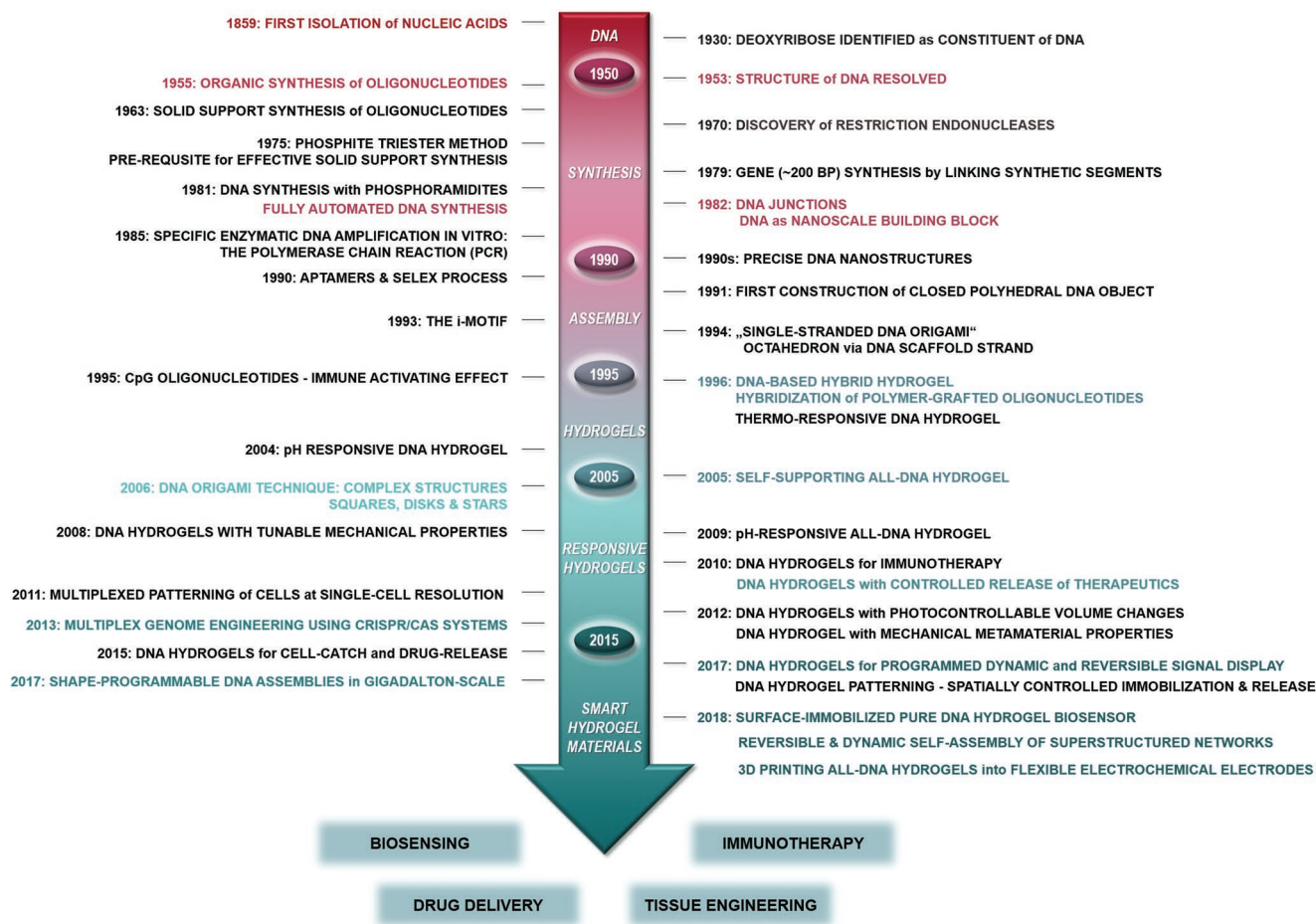FUNCTIONAL
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Figure 1. Timeline of notable discoveries and developments in DNA technology and their relevance for DNA-containing hydrogels.

The selected cross-linkers in all-DNA hydrogels strongly influence the material properties of the resulting gels. Hydrogels made by hybridization of sequences with complementary sticky ends can undergo sol–gel transitions upon heating,[35] or by pH-changes[36] and they can be reversibly cycled through liquid–solid phase transitions. In contrast, gels made by enzymatic ligation are covalently cross-linked networks that do not undergo reversible sol–gel transitions.[34] In addition, the application of enzymatic DNA polymerization techniques can result in gels with metamaterial properties exhibiting solid-like behavior when immersed in water and liquid-like behavior after water removal.[37] Furthermore, after complete deformation due to removal of water, the material shows shape-memory behavior as it recovers its original shape upon addition of water.[37] Similarly, Chen and Romesberg used PCR amplification in combination with two specially synthesized forward and reverse bottlebrush primers to form DNA hydrogels that exhibit hierarchical architectures.[39] These gels have been rendered catalytically active by incorporating streptavidin-modified horseradish peroxidase after amplification of biotinylated bottlebrush primers, which could be attractive for encapsulation of specific guest molecules without loss of protein function.[39]

Although all-DNA-based hydrogels reveal many unique properties, there are still several limitations that need to be overcome to facilitate their usage in biomedicine. First of all, very few functional groups are available in DNA, which makes further chemical modifications rather challenging and limits customization of the functional properties of the hydrogel. In addition, some intrinsic limitations of DNA, such as its highly negative net charge, limited stability, and high synthesis costs currently prevent upscaling that would be necessary for in vivo biomedical applications. Consequently, only very few examples have been reported, in which all-DNA hydrogels have been used beyond proof-of-concept demonstrations, e.g., in 3D printing.[40,41]

2.2. Oligonucleotides as Cross-linkers

Some of these limitations may be overcome by combining hydrophilic polymer scaffolds with short DNA sequences acting as supramolecular cross-linking agents.[26,29,42] Such hydrogels utilize both the smart and programmable features of the DNA components as well as the chemical flexibility, stability, and accessibility of hydrophilic polymers to optimize functional performance. In this way, bulk materials are more readily available and several of the unique material characteristics of DNA are retained.

In 1996, Nagahara and Matsuda reported the first DNA-containing polymer hydrogels that were based on short DNA
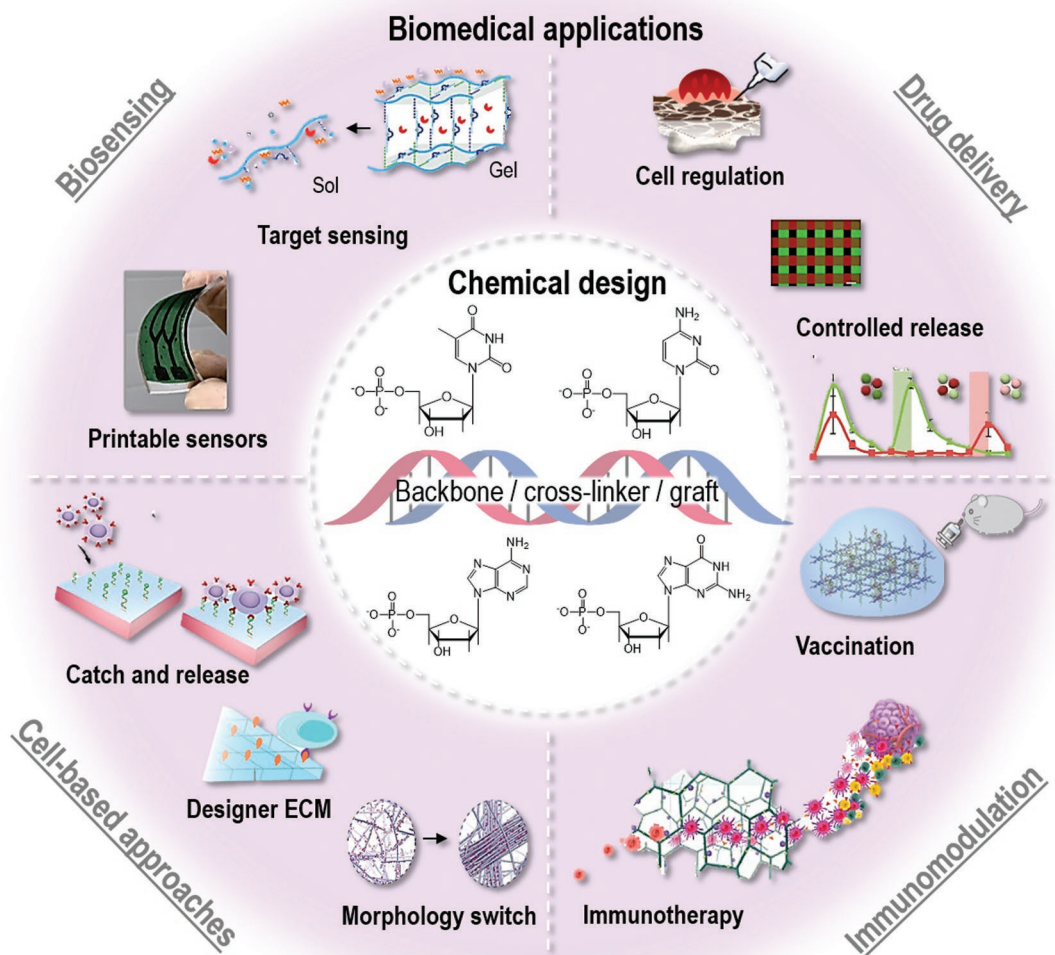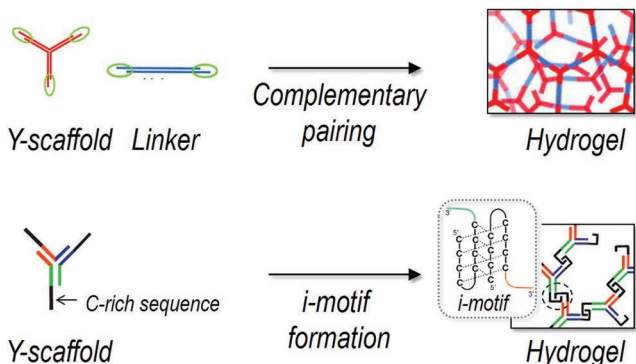
Figure 2. Overview of the manifold applications of DNA-based hydrogels in the biomedical field. Highlighted examples include biosensing, drug delivery, immuno-modulation, and cell-based approaches. Printable sensors: Adapted with permission.[41] Copyright 2018, Wiley-VCH. Target sensing: Adapted with permission.[107] Copyright 2017, Royal Society of Chemistry. Cell regulation: Adapted with permission.[49] Copyright 2017, Wiley-VCH. Controlled release (top): Adapted with permission.[143] Copyright 2018. American Chemical Society. Controlled release (bottom): Adapted with permission.[73] Copyright 2012, American Chemical Society. Vaccination: Adapted with permission.[149] Copyright 2015, Elsevier. Immunotherapy: Adapted with permission.[162] Copyright 2018, American Chemical Society. Morphology switch: Adapted with permission.[180] Copyright 2018, American Association for the Advancement of Science. Designer ECM: Adapted with permission.[183] Copyright 2016, Wiley-VCH. Catch and release: Adapted under the terms of the Creative Commons Attribution 4.0 International License.[76] Copyright 2015, The Authors.

sequences grafted to a poly(acrylamide) polymer chain.[43] The authors demonstrated two pathways of achieving gelation: In so-called Type I hydrogels, a soluble DNA sequence hybridized with two other DNA strands attached to a polymer backbone, thereby inducing gelation (**Figure 4**).[43] In contrast, Type II hydrogels had complementary DNA strands attached to the polymer chain and gelation occurred in situ without the addition of external gelators (Figure 4).[43]
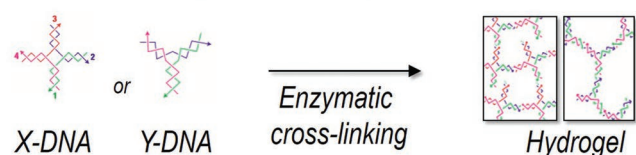
Following this seminal work, various DNA-polymer hybrid materials have been prepared with customized sol–gel transitions that are externally controlled by complementary DNA strands.[44–46] Complex DNA cross-linking elements such as

Y-shaped DNA,[47–49] i-motifs,[50] or DNA mismatch elements[51] have also been introduced into polymeric hydrogels (Figure 4, **Table 1**). Y-shaped DNA provides hybridization, gelation, and functionalization of hydrogels in one step, where the DNA linkages could be severed by heat, enzymes, or mechanical stress thus yielding gels with attractive multi-responsiveness and thixotropic behavior.[47–49] Light-responsiveness has been achieved by incorporating an azobenzene-modified ssDNA into an acrylamide hydrogel inducing macroscopic volume changes of the dynamic hydrogel upon alternating irradiation with visible and UV light.[52] Furthermore, by exploiting the possibility to mismatch the sticky-end sequences, hydrogels with tunable mechanical and thermal

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a) Hybridization

Y-scaffold | Linker → *Complementary pairing* → Hydrogel

Y-scaffold | ← C-rich sequence → *i-motif formation* → i-motif | Hydrogel

b) Enzyme catalyzed assembly

X-DNA | or | Y-DNA → *Enzymatic cross-linking* → Hydrogel

c) Entanglement

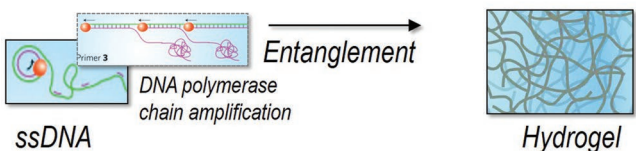ssDNA → *DNA polymerase chain amplification* → *Entanglement* → Hydrogel

Figure 3. All-DNA hydrogels formed by a) hybridization of Y-shaped DNA[35] and i-motifs,[36] b) enzyme-catalyzed assembly,[34] and c) entanglement of long DNA chains formed via DNA polymerase chain amplification.[37] a) Adapted with permission.[35,36] Copyright 2009 and 2010, Wiley-VCH. b,c) Adapted with permission.[34,37] Copyright 2006 and 2012, Springer Nature.

properties were obtained.[51] Mismatches refer to regions within the sticky-ends of the DNA cross-linker that are partially complementary to the ssDNA grafted on the polymer backbone.[51]

Moreover, (multi)-stimuli-responsive gels,[42,50,53–63] logic gates,[64] and shape-memory[42,56,62,63] hydrogels have been reported based on DNA cross-linkers by additionally implementing DNA motifs like i-motifs, guanine quadruplex units, or aptamers (Figure 4c and Table 1). Briefly, guanine quadruplexes are formed from guanine-rich nucleic acid sequences, where four guanine bases associate through hydrogen bonding to adopt a square planar structure, which is stabilized by ions like K^+. Aptamers (*Latin from aptus—fit, Greek from meros—part*) are single-stranded DNA or RNA sequences that can selectively bind various substrates such as small molecules,[65–67] proteins,[68–74] or even cells,[75–77] with affinities comparable to antibodies.

The switching of multi-responsive hydrogels can be achieved by several cooperative stimuli-responsive DNA cross-linking motifs, each dictating the hydrogel properties. In this way, controlled dissociation and reassembly occurs, transitioning the hydrogel between different states such as stiff or quasi-liquid states.[42] These gels can even function as logic gates,

i.e., dissociation only occurs in the presence of two distinct target molecules but they remain intact if only one or none of the target molecule is present.[64] In shape-memory hydrogels, e.g., via the i-motif bridging units, cooperative cross-linking of nucleic acid-functionalized polymer chains has been reported.[62] Thereby, the two cooperative cross-linking strategies retain a certain hydrogel shape. Upon dissociation of one cross-linker, a "memory code" in the quasi-liquid state is provided by the other, still intact cross-linking unit allowing reorganization into the original shape.[42,62]

2.3. Oligonucleotides as Bioactive Groups or as Tags for Functionalization

Hydrogels incorporating DNA segments as functional, bioactive elements and not as structural components have also been explored for various biomedical applications.[49,68,70–77] The presence of DNA as a bioactive group in these hydrogels typically does not significantly alter the mechanical properties of the gel, but rather imparts important interactions with therapeutically relevant molecules[49,68,70–74] or cells.[75–77]
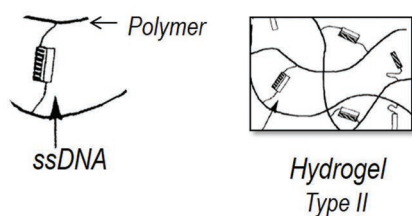
Functionalization of DNA hydrogels is straightforward, only requiring introduction of short DNA sequences comprising the desired functionality during[47–49] or after gelation.[78] These DNA tags hybridize complementary DNA strands at the polymer backbone or within the already formed hydrogel (Figure 4d). Similarly, phosphoramidite derivatives provide a synthetic route of oligonucleotides that are functionalized with a methacryl group at the 5' end DNA (also called acrydite-modified DNA) and can subsequently be added during the gel-forming polymerization reaction of, i.e., acrylamide.[79–82]

Previously, DNA strands have been modified with reactive groups such as amine, carboxyl, thiol, or alkyne groups, as well as azobenzenes as light-sensitive switches, bioactive peptides, or proteins.[83,84] Moreover, therapeutically active compounds[49] or biomimetic signals[9] have been attached to the hydrogels, making them responsive toward enzymes or enabling strand displacement for controlled release of functional molecules. A potential drawback of the post-functionalization method of already formed gels is the need to infiltrate the hydrogel with the functionalized DNA tags, which could be sterically or electrostatically hindered, particularly if larger DNA structures need to be incorporated.
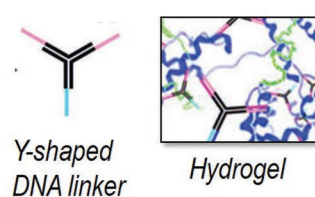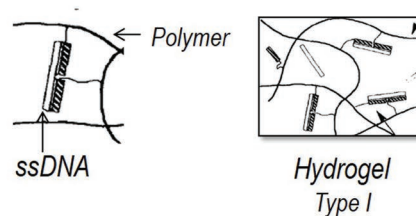
In addition to the already mentioned reactive functionalities and bioactive entities, aptamers have gained significant interest because of their high specificity toward a wide range of biological target molecules and the opportunity to selectively introduce functional molecules to the hydrogel (Figure 4e). Compared to antibodies, aptamers can have significantly smaller molecular weights, and they can be easily tailored for different targets, which makes them attractive as components in bioassays[85,86] or as therapeutics for treatment of diseases such as eye disorders, thrombosis, vascular diseases, and cancer.[68] Aptamer sequences with high target-specific binding affinity values are generated by an in vitro selection process called *systematic evolution of ligands by exponential enrichment*.[87,88] One drawback of the application of aptamers
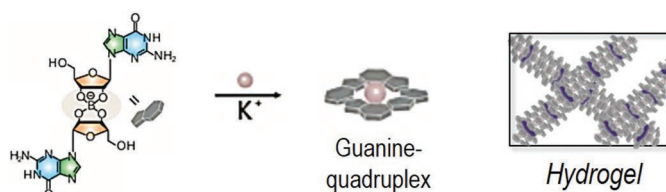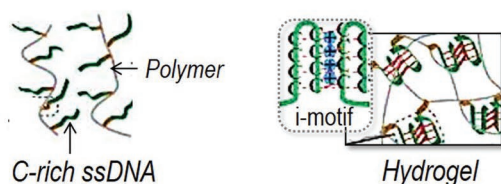
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Cross-linking

a) Direct DNA hybridization

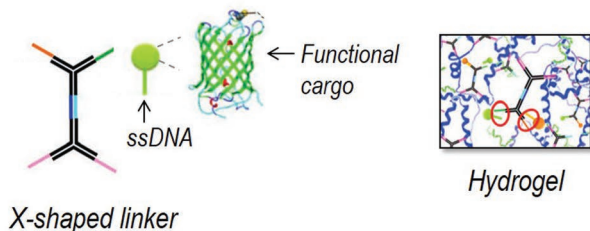### b) ssDNA linker units

c) Structure elements

Functionalization

d) Modified DNA sequences
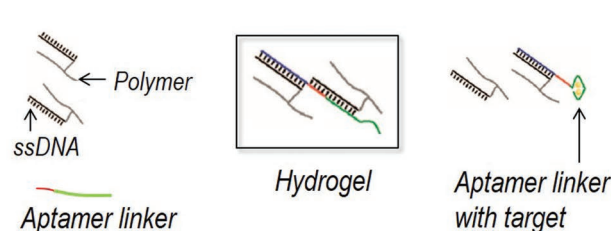
e) Aptamer sequences

Figure 4. Oligonucleotides as hydrogel cross-linkers by a) direct DNA hybridization,[43] b) ssDNA linker units[43,48] including DNA origami linkers, or c) structure elements like i-motifs[50] or guanine quadruplex units.[41] Functional hydrogels by d) hybridization of modified DNA sequences[48] or by e) implementing aptamer sequences into the hydrogel.[57] Aptamers can bind various biological target structures. a) Adapted with permission.[43] Copyright 1996, Elsevier. b) Left: adapted with permission.[43] Copyright 1996, Elsevier. Right: adapted with permission.[48] Copyright 2014, Royal Society of Chemistry. c) Left: Adapted with permission.[50] Copyright 2014, Wiley-VCH. Right: Adapted with permission.[41] Copyright 2018, Wiley-VCH. d) Adapted with permission.[48] Copyright 2014, Royal Society of Chemistry. e) Adapted with permission.[57] Copyright 2008, American Chemical Society.

is their low cellular uptake due to their high negative charge density[89] as well as their limited stability in the presence of DNA degrading enzymes that are typically present in cells.[90] However, there are promising strategies to optimize their function, reduce their susceptibility to nuclease degradation, and improve the stability of aptamers to broaden their scope for biomedical applications.[68,90,91]

In the following, DNA hydrogels that were prepared by the already discussed design strategies and based on the introduced structural components are reviewed in view of their biomedical applications. We mainly focus on applications of DNA hydrogels as matrices for biosensing, as vehicles for drug delivery and immunomodulation, as well as their applications for cell-based approaches such as tissue engineering.

2.4. Critical Comparison with Non-DNA Cross-linkers in Supramolecular Hydrogels

While DNA-containing hydrogels are an emerging material for biomedical applications, competing systems consisting of, e.g., biopolymers and peptides can also offer certain advantages. In the following, we critically compare a limited number of hydrogel systems that share certain aspects of the DNA-containing hydrogels discussed in this review. Similar to DNA, peptide sequences can also participate in (self-)recognition events, that lead to, i.e., the formation of α-helical or β-sheet structures. These features have been used to generate noncovalently cross-linked hydrogels by using short β-sheet-forming peptide-grafts as cross-linking sites to afford hybrid hydrogels with inner fibrillary structures.[92–94] Other

Table 1. DNA-motifs used for cross-linking of DNA hybrid hydrogels. While reversible transitions between DNA-based polymer hydrogels and DNA-polymer solutions or quasi-liquid states can be implemented by employing different triggers like strand displacement, target-responsiveness, pH, and different ions for G-quadruplexes, or metal ions, they also offer the potential to construct smart shape-memory hydrogels when combining orthogonal stimuli.

DNA-motif	Stimulus	Polymer	Hydrogel features	Ref.
DNA duplex	Strand displacement	PAAm[a]	Sol–gel transition	[42,61]
		DNA	Shape-memory behavior and sol–gel transition	[42]
				[194]
Aptamer	Target-responsive, e.g., ATP, adenosine	PAAm, DNA	Target-induced sol–gel formation Tunable mechanical properties	[53,57,58,194]
				[59]
Y-DNA	Heat, enzymes, mechanical stress	Protein-based	Enzyme-controlled degradation Sol–gel transition	[47–49]
i-Motif	pH	PNIPAM[b]	pH 5: gel	[42,50,62]
	pH, Ag^+/cystamine	PAAm	pH 8: sol	
		Acrylamide	Shape-memory behavior	[63]
Azobenzene-ssDNA	Light	Acrylamide	Volume changes after irradiation with Vis and UV light, Tunable mechanical properties	[52]
Mismatches	DNA linker sequence	Polypeptide	Tunable mechanical and thermal properties	[51]
G-Quadruplexes	K^+/crown ether	Acrylamide, Carboxymethyl cellulose	K^+: gel	[42,54,195]
	Pb^{2+}/DOTA, Sr^{2+}/kryptofix	Acrylamide	Crown ether: sol	
			Shape-memory behavior	[42,195]
			Nanochannel with multiple gating features	[60]
			Shape-memory behavior	[63]
Metal-ion/ligand	Redox environment	PAAm, Carboxymethyl cellulose	Tunable mechanical properties, shape-memory effects	[56]
	Ag^+/cysteamine	Acrylamide	Ag^+: gel	
			Cysteamine: sol	[55]
			Tunable mechanical properties	

[a] Poly(acrylamide); [b] Poly(N-isopropylacrylamide).

examples include the coiled-coil motif and collagen mimicking peptides which, based on the simplicity and regularity, have been extensively studied in this context as well.[95–100] The structure formation of coiled-coil and collagen mimicking peptides is affected by the number of repeat units, pH, ionic strength, temperature, and concentration. Consequently, a variation of the peptide or graft copolymer concentrations affect certain hydrogel characteristics (e.g., gelation time, morphology, stiffness, melting point temperature) and can be used to introduce stimulus responsive behavior.[98,101,102] Recently, these systems have been extensively reviewed and there has been much progress to design such hydrogels for biomedical applications.[101,103] One of the major advantages of these peptide-containing hydrogels is their promising scalability compared to the significant costs still associated with DNA synthesis. In terms of mechanical properties, DNA-containing hydrogels often do not outperform alternative systems without DNA. However, DNA reveals exceptional recognition properties, which drastically increases the programmability of DNA-containing materials

and enhances their functional applications in particular for biosensing, drug delivery, and tissue engineering as discussed in the next sections.

3. Biomedical Applications of Nucleic Acid-Based Hydrogels

3.1. DNA-Based Hydrogels for Biosensing

The qualitative and quantitative detection of various analytes in vitro and in vivo is vital in many biomedical fields spanning clinical diagnosis and point-of-care testing to detecting trace targets in personalized healthcare, environmental monitoring, and food safety.[104,105] Particularly, portable and visual detection methods provide the technology for devices that are fast, simple to operate, and do not require special instruments.[104,105] In this context, target-specific stimuli-responsive DNA hydrogels could serve as a portable, sensitive, selective, and low-cost biosensor platform.

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Typical design strategies of biosensor hydrogels employ a synthetic polymer as the scaffold and an analyte-binding, functional DNA cross-linker as the sensor. The cross-linker dissociates only after recognition of the respective target compound and reports its presence by inducing changes in hydrogel swelling volume, mass, mechanical properties, or by the release of an encapsulated cargo generating a readout signal.[106,107] To date, different target-responsive DNA hydrogels have been reported[104–106] for the detection of ions,[81,108,109] small molecules,[53,57,64] proteins,[110] viruses,[111] or toxins.[112,113] In certain cases, the readout from these DNA-based devices is even visible to the unaided eye, without the need for sophisticated equipment and is also preserved in biological matrices including urine, serum, and lacrimal fluid.[53,110,111,114,115] This feature has been exploited for specific recognition of thrombin, which is relevant in the context of glomerulonephritis, an inflammation of the kidney's filtering systems which occurs in certain cancers, diabetes, hepatitis, or the autoimmune disease *Systemic Lupus Erythematosus*.[110,111,116] As the signal readout is one of the central differentiating elements of biosensors, the detection of various biomedical targets and metabolites by colorimetric, volumetric, or conformational interconversion as well as the development of electrochemical readout strategies for printable sensors are highlighted in the following sections.

Recently, Mao et al. described the sensitive and visual detection of different targets employing a functional DNA cross-linked hydrogel (**Figure 5**a).[107] The hydrogel was based on aptamers or DNAzymes, DNA oligonucleotides catalyzing certain chemical reactions, as a target-responsive unit that was incorporated into hydrogels able to bind ions or small molecules. In addition, glucoamylase, an enzyme that catalyzes the digestion of amylose to glucose, was encapsulated for both molecular recognition and signal amplification.[107] In these gels, gold nanorods served as a multicolor readout circuit for monitoring the target concentration, e.g., Pb^{2+}, with high sensitivity. Lead concentrations of about $50–100 \times 10^{-9}$ M were detected with this system by simple visual inspection. Thus, portable hydrogels with DNAzyme–substrate complexes as cross-linkers of a DNA-grafted polyacrylamide polymer backbone even allowed visual quantification of lead ion concentrations in aqueous solutions.[107] Important biomarkers such as adenosine,[57,105,117] which acts as a signaling molecule in the peripheral and central nervous system and also represents a cancer biomarker for monitoring disease progression,[105] as well as thrombin[57] have been detected by similar colorimetric assays. Colorimetry is the detection method of choice in most DNA hydrogel biosensors as it allows the straightforward detection of target analytes such as metal ions[108,117] and small molecules,[105,115,117,118] e.g., for environmental monitoring, forensic analysis, detection of drug misuse, and medical diagnosis. However, this technique often provides only semiquantitative analysis and relatively low sensitivity.[119]

To overcome these limitations, Liu et al. used a molecular imprinting approach to form hydrogels that undergo macroscopic volumetric shrinkage in the presence of target proteins.[110] Such hydrogels were formed by aptamers carrying a polymerizable methacrylamide group together with bifunctional aptamer–protein complexes as small cross-linkers. In this way, the proteins thrombin and PDGF-$\beta\beta$, which are released during blood clotting and serve as biomarker for angiogenesis and atherosclerosis, were imprinted into the gel. The resulting gels showed remarkable sensitivity down to femtomolar concentrations of PDGF-$\beta\beta$. In addition, the dried gels were stable for extended time periods and could be re-hydrated on demand.[110]
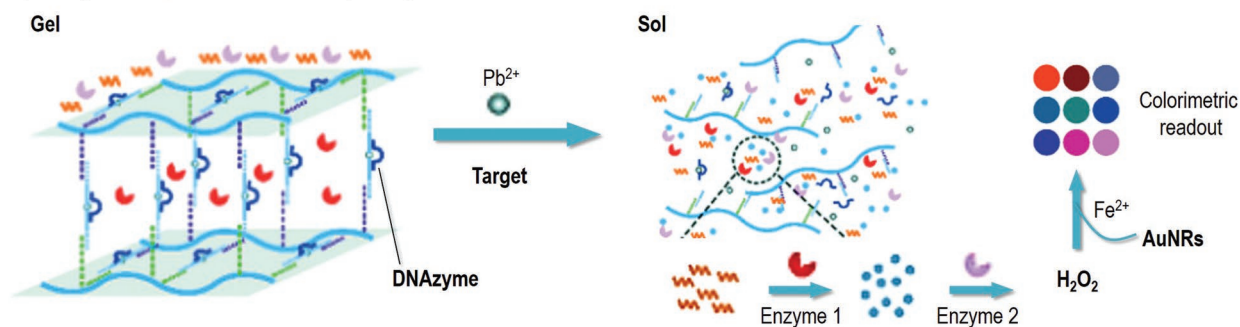
The development of surface-immobilized DNA hydrogels as printable sensors and for bioelectronics has been explored recently where applications in the fields of personalized medicine and clinical diagnosis are anticipated.[41,119,120] Mao et al. reported the first surface-immobilized pure DNA hydrogel biosensor (Figure 5b).[120] The soft DNA hydrogel scaffold was synthesized via a surficial primer-induced strategy on a transparent electrode, allowing both colorimetric and electrochemical detection. The resulting immobilized 3D DNA hydrogel scaffold was employed to capture enzymes, providing a stable catalytic system for biosensing. On account of the molecular sieving effect and the good swelling/de-swelling properties, direct sensitive detection of targets such as bilirubin in serum samples was successfully demonstrated. Furthermore, fast regeneration of the material and preservation of the catalytic activity after a few cycles was achieved.[120] The observed low detection limit of bilirubin of 32×10^{-9} M was proposed to allow for applications in the diagnosis of jaundice.[120]

Zhong et al. reported on a DNA hydrogel-based, highly selective and sensitive sensor for Pb^{2+} detection (Figure 5c), which was based on conformational interconversion that induces changes in the catalytic activity with impressive responses between 1×10^{-12} and 50×10^{-9} M. The hydrogel was constructed from a guanosine-borate monoester with subsequent K^+-templated assembly into guanine quadruplexes (G_4-DNA, as discussed in Section 2.2, Figure 4c). Guanine quadruplexes incorporate hemin and display enzyme-like activity.[40] The sensing event is based on Pb^{2+} substituting K^+ within the complex and subsequently inducing a conformational interconversion to more compact structures that trigger hemin release.[40] This subsequent loss in catalytic activity was detected as changes in the absorbance that contribute to the high sensitivity of the sensor.[40] The authors suggest that the system provides a novel platform for sensing, biomolecular computation, and drug delivery, due to its facile construction combined with long-term stability (up to 28 days), high sensitivity, and selectivity.[40]
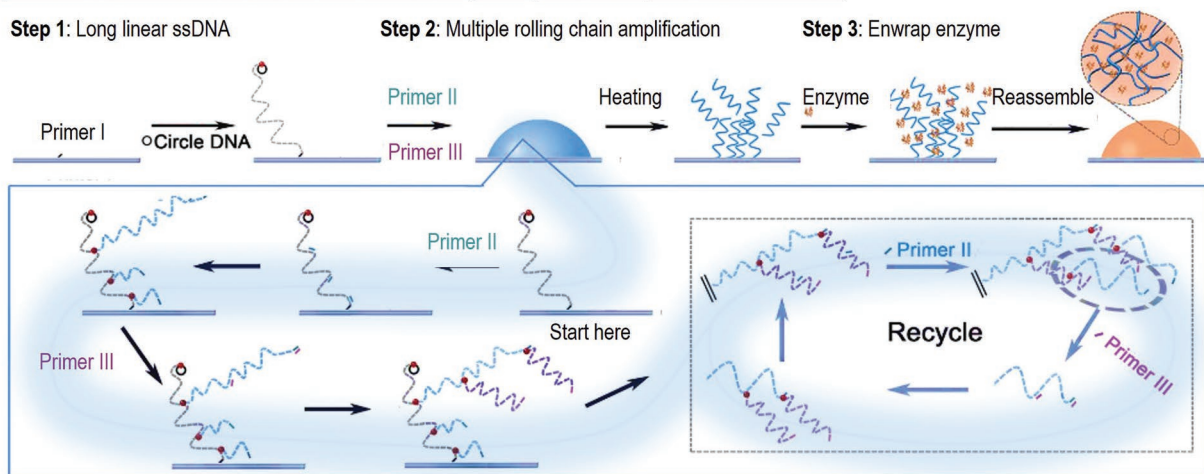
The immense potential of this setup for flexible next-generation sensors and bioelectronics was also underlined by the in situ fabrication of a semiconducting hydrogel as a glucose biosensor (Figure 5d).[41] This biosensor was fabricated by loading the enzyme glucose oxidase into the DNA hydrogel as a catalytic matrix for the oxidation of aniline and the in situ deposition of polyaniline. In this way, the biosensor was directly printed into a flexible electrochemical electrode. This detection system revealed excellent electrochemical properties, long-term and cycle stability and was compatible with 3D printing, rendering it a promising material for flexible electrochemical applications such as the development of self-regulated insulin delivery devices.[41]

Another DNA hydrogel-based electrochemical biosensor was designed for the detection of microRNA that is specific to lung cancer.[119] The hybrid hydrogel was immobilized on an indium tin oxide/polyethylene terephthalate (ITO/PET) electrode, and
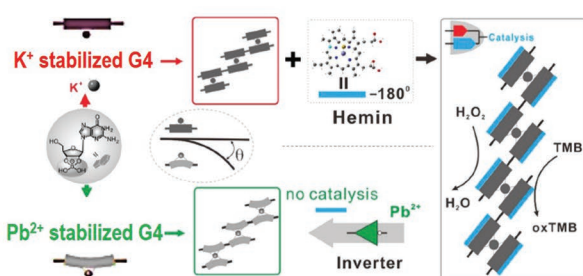
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a) Target-responsive DNA hydrogel based on strand-displacement

b) Printable surface-immobilized DNA hydrogel with spatial definition

c) Target-responsive DNA hydrogel based on catalytic interconversion

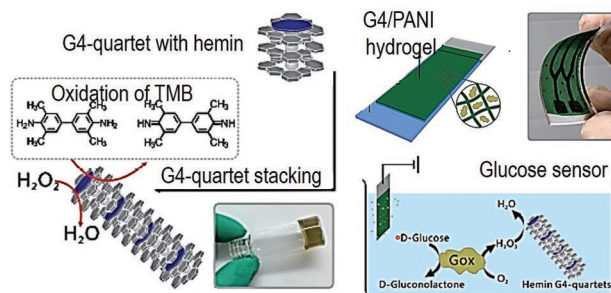### d) Flexible biosensors from conductive material

Figure 5. DNA-based hydrogels for biosensing. a) Target-responsive readout-unit release via gel–sol transition for colorimetric readout. The target-responsive strand can be an aptamer resulting in strand displacement and hydrogel collapse. Alternatively, a DNAzyme is activated by the target to cut the cross-linking strand. Enzymes like glucose amylase can be used as readout-unit catalyzing colorimetric reactions for visual detection. AuNRs served as a multicolor substrate. Adapted with permission.[107] Copyright 2017, Royal Society of Chemistry. b) Printable surface-immobilized DNA hydrogels with high spatial definition. Surface-immobilized 3D DNA hydrogels were constructed on a transparent ITO electrode using a surficial primer-induced strategy for both colorimetric and electrochemical readout. For biosensing, the hydrogel can wrap enzymes for detection of, e.g., bilirubin in serum.[120] Adapted with permission under the terms of the Creative Commons Attribution-NonCommercial 3.0 Unported license.[120] Copyright 2018, The Authors. Published by Royal Society of Chemistry. c) Target-responsive DNA hydrogel based on catalytic interconversion resulting from conformational transition via cation substitution. The hydrogel comprised a G4-quartet motif with hemin and complexed K^+ ions forming a catalytically active material. Cation substitution with Pb^{2+} forms more compact structures and simultaneous loss of hemin is followed by drastic change in the catalytic activity of the hydrogel where the H_2O_2/3,5,3,5-tetramethylbenzidin (TMB) oxidation was chosen as a model reaction. Adapted with permission.[40] Copyright 2018, American Chemical Society. d) Flexible electrochemical biosensors. Enzyme-mimicking hydrogel matrix catalyzes the in situ oxidation of aniline yielding the deposition of polyaniline on G-quartet nanofibers. Detection of glucose was demonstrated by loading glucose oxidase enzyme (GOx) into the hydrogel. Adapted with permission.[41] Copyright 2018, Wiley-VCH.

DNA-grafted polyacrylamide cross-linked by ferrocene-tagged DNA served as a recognition probe.[119] Upon hybridization of the recognition probes with the target miRNA, the hydrogel dissociated, resulting in a loss of ferrocene tags and, hence, a reduction in measurable current.[119] This biosensor was able to detect concentrations as low as 5×10^{-9} M (1 pmol) and showed a linear readout ranging between 10×10^{-9} and 50×10^{-6} M. It was highly sensitive, and showed good stability and selectivity. Furthermore, it can be applied toward a diversity of other targets as the recognition probe has a flexible sequence design.[119] These strategies indicate that DNA hydrogels could be integrated into surface biosensing devices, thereby, broadening hydrogel applications in the field of diagnosis, personalized medicine, and environmental monitoring. Future challenges lie in the development of sensing devices that have improved stability and are operational under a wide range of environmental conditions. In particular, the ability to accurately detect and quantify trace amounts of analytes in complex body fluids still represents a great challenge. In addition, current DNA hydrogel-based sensors are quite costly preventing their application as single-use devices. Therefore, either the reusability of these sensors needs to be significantly improved, or the production costs have to be substantially lowered. With the advent of personalized medicine, the need for individualized bioanalytics is gaining much attention. We anticipate that if the abovementioned challenges can be addressed, DNA hydrogel sensors will offer great potential in future personalized medicine as implantable sensors or for in vivo diagnostics.

3.2. DNA-Based Hydrogels for Drug Delivery Applications

One major concern in drug delivery is the capability to store and release drug molecules or therapeutic proteins in a controlled spatio-temporal fashion, i.e., based on changes in pH, temperature, the presence of enzymes, or light.[121] In this section, we discuss the customization of DNA hydrogels for various drug delivery applications to address tumor tissue or bone cells locally. First, examples dealing with hydrogels capable of releasing small molecules or therapeutic proteins are summarized. Then, more sophisticated drug depots that can target specific cells and release their cargo in response to changes in their environment or due to other external triggers are discussed. Finally, we present selected examples of such gels capable of storing and delivering therapeutic proteins in a spatio-temporal fashion in the context of different diseases such as osteoporosis.

Early hydrogel formulations based on enzymatically cross-linked DNA hydrogels lacked a specific ability to retain target molecules within the network, and their release kinetics were determined predominantly by diffusion parameters.[34] In recent years, DNA hydrogels have become more advanced and can now release drugs in a controlled fashion to improve their therapeutic performance as drugs are made available over prolonged periods of time.[70,73,122]
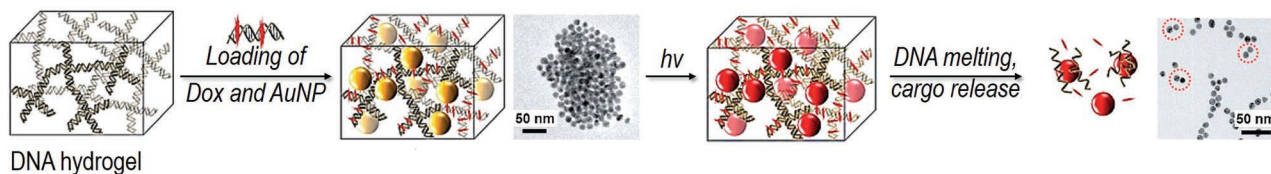
Hydrogel formulations are of particular interest for the controlled and local delivery of anticancer drugs to improve their efficacy and reduce off-target side effects.[123,124] The most common cancer treatments involve local surgery or radiation therapy, as well as systemic approaches including chemotherapy,

hormonal therapy, and antibody treatments.[125,126] Conventional chemotherapy based on cytotoxic small molecule drugs is considered a systemic treatment option for the elimination of cancerous cells and tissues, with the inherent limitation that these cytotoxic drugs often lack specificity toward cancerous cells versus healthy ones.[69,127] After systemic administration, drugs are distributed throughout the body and often only a small fraction reaches the tumor site, whereas organs and nonmalignant tissues are frequently also negatively affected causing severe side effects.[69,127] Also, fluctuating drug levels represent a concern as most cytotoxic drugs are released directly after administration causing sharp peaks and subsequent decline in the drug concentration, yielding inadequate therapy and severe side effects.[128] Consequently, there is a strong demand for targeted and controlled release technologies. Hydrogels, with tunable sustained release characteristics and injectable formulations, fuel legitimate hopes to improve drug efficacy and reduce the impact on healthy cells or tissues.
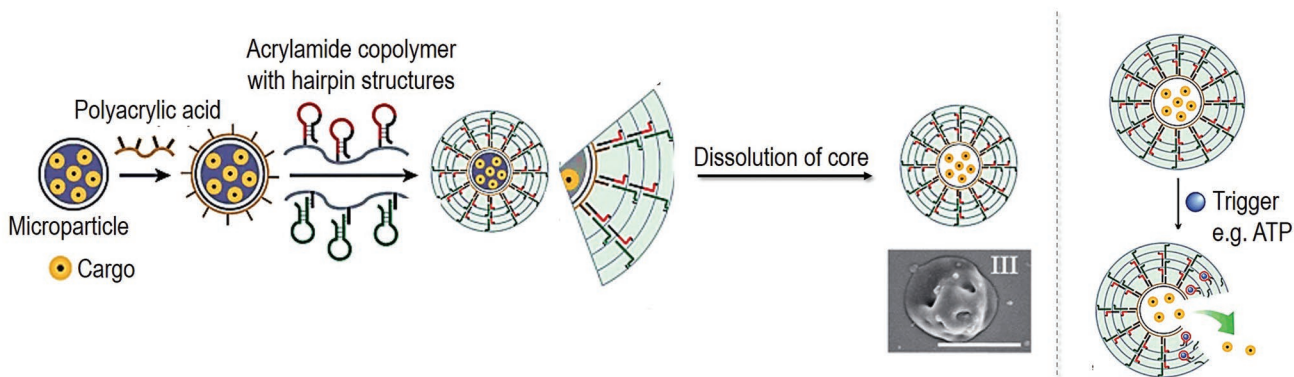
The recently reported concept of drug-integrated DNA Trojan Horses aims at overcoming the limitations and severe side effects of classical therapy by systemic injection, which is characterized by strong off-target effects. With DNA Trojan Horses, an enhanced cellular uptake, higher accumulation of the drug in the target tissue, and a more efficient, localized release aim to achieve greater antitumor effects.[129] Cytotoxic nucleoside analogs, such as fluoroxidine, which are an important class of anticancer drugs, were directly integrated into DNA via solid-phase synthesis. In a hierarchical self-assembly process, these drug-containing single-stranded DNAs can hybridize into Y-shaped motifs, which then assemble into nanogels with a precise drug loading ratio. These nanogels resemble spherical nucleic acid-like architectures constituting novel Trojan Horses for drug delivery and anticancer treatment.[129] In vitro experiments showed that the fluoroxidine-containing nanogels provided rapid cellular uptake and effective drug release in the presence of DNases, resulting in an enhanced anticancer effect compared to the free drug as control.[129]

Another strategy for efficient cancer treatment employs light-triggered drug release by incorporating gold nanoparticles (AuNPs)[130] or gold nanorods (AuNRs)[124] into DNA hydrogels in combination with anticancer drugs such as Doxorubicin (Dox). Song et al. reported a hydrogel that was assembled by enzymatic ligation of X-shaped DNA building blocks composed of three sticky arms for cross-linking and one other arm.[124,130] Employing simple electrostatic interactions between the negatively charged DNA and the positively charged gold substrates, AuNPs or AuNRs were loaded into the hydrogel, while Dox was simultaneously incorporated via intercalation into the DNA completing the drug delivery system (**Figure 6**a). Release of Dox was achieved through a photothermal effect after laser excitation of the AuNPs, which led to a heat shock and subsequent disassembly of the whole construct. The cytotoxicity of this system was found to be boosted, illustrating the synergy between photothermal and chemo-therapeutic approaches in cancer treatment. This led to a reduction in the necessary dosage of either the laser power or drug concentration providing opportunities to reduce side effects when applied in photothermal therapy.[130] Based on the closely assembled AuNPs with strongly coupled plasmon modes, these hydrogels could potentially also be

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a) Triggered drug-release and photothermal therapy

b) Triggered cargo-release from DNA-based hydrogel microcapsules

c) Triggered drug-release after targeted cancer cell recognition
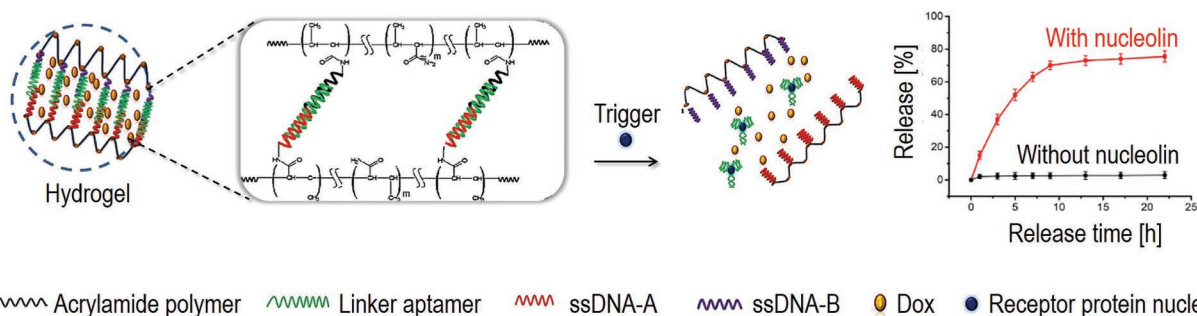

Figure 6. DNA-based hydrogels for drug delivery applications in anticancer therapy. a) DNA-based hydrogels as delivery vehicle for AuNPs and anticancer drugs combining triggered drug release and photothermal therapy. The DNA hydrogel is degraded after excitation-induced heat generation, which disperses the AuNPs. Co-loading of the anticancer drug Dox facilitated light-controlled cargo release from the hydrogel, which is attractive for, i.e., computer tomography (CT), imaging contrast, or radiosensitization. Adapted with permission.[130] Copyright 2018, American Chemical Society. b) Triggered release of therapeutic molecules from stimuli-responsive DNA hydrogel microcapsules. Polymer chains were equipped with nucleic acid hairpin structures as well as single-strand tethers as hybridization and recognition units to achieve substrate-loaded stimuli-responsive hydrogel-based microcapsules. Microcapsules were loaded with $CaCO_3$ microparticles and the respective substrates, i.e., Dox, and functionalized with nucleic acid promoter units, which induce hybridization chain reaction (HCR) upon presence of the hairpin-modified chains. A hydrogel coating was formed, which, after the dissociation of the $CaCO_3$ cores, yields substrate-loaded hydrogel microcapsules. Stimuli-responsiveness was implemented into the microcapsule by caged aptamer sequences recognizing, e.g., ATP. In the presence of ATP, the duplex-caged aptamer sequences are released and new substrate–aptamer complexes were formed thus releasing the Dox cargo molecules. Adapted with permission under the use of the Creative Commons Attribution 3.0 Unported license[131] Copyright 2017, the Authors, published by Royal Society of Chemistry. c) Triggered cancer drug release after targeted cancer cell recognition by using an aptamer-functionalized hydrogel system. Aptamer sequences are used as cross-links of DNA-grafted acrylamide polymers. The aptamers bind specifically to nucleolin receptors that are overexpressed in the membrane of many tumor cells. The enhanced affinity of the aptamer toward the nucleolin receptors effects changes of the aptamer conformation thus reducing the cross-linking density and leading to hydrogel dissolution, releasing the encapsulated drug Dox. Adapted with permission.[123] Copyright 2015, Elsevier.

exploited as contrast agents in CT imaging or in radiosensitization competing against large AuNPs, with sizes of hundreds of nanometers that are typically used and exhibit increased toxicity, thus limiting their applications.[130]

Another attractive example was developed by Liao et al. who applied chemical, rather than physical triggers for drug release. They reported on microcapsules consisting of stimuli-responsive DNA hydrogels that released drugs in the

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presence of specific ligands based on aptamer technology (Figure 6b).[131] In order to prepare the construct, first, drug-loaded $CaCO_3$ microcapsules were coated with a layer of poly(allylamine hydrochloride) and subsequently with poly-acrylic acid. Finally, the construct was functionalized with nucleic acid promoter units. The promoter units induced a hybridization chain reaction in the presence of nucleic acid hairpin structures leading to a cross-linked DNA hydrogel that coats the particles. Stimuli-responsiveness was implemented by incorporating ligand-specific aptamer sequences within several hairpin units. These microcapsules were able to release their cargo drug, Dox, in the presence of adenosine triphosphate (ATP) due to its interaction with the aptamer cross-links leading to the dissociation of the cross-linking nucleic acid duplex units and partial separation of the micro-capsule shells. ATP is overexpressed in cancer cells making it an interesting trigger for site-selective release systems.[131]

This strategy was further expanded to metal organic framework nanoparticles (NMOFs) that are prepared as carriers for cargos like cancer drugs. Here, duplex DNA-capped drug-loaded NMOFs have been developed to introduce stimuli-responsiveness but are associated with shortcomings such as limited loading degrees and nonspecific drug leakage. To address these issues, DNA hydrogel-coated NMOFs were introduced for controlled drug release.[132–134] The hydrogel-coated NMOFs revealed substantially higher drug loading compared to NMOFs locked by duplex DNA capping units.[133,134] In particular, the hydrogel-coated NMOFs do not show nonspecific drug leakage while exhibiting high cytotoxicity (40% within a time interval of 3 days after treatment) and selectivity toward MDA-MB-231 cancer cells.[132]

In addition, small triggering molecules, i.e., metabolites, or receptor proteins can bind and change the aptamer conformation inducing a switch from the DNA bound to an unbound state (Figure 6c).[123] This principle was employed for cancer cell recognition and targeted drug delivery of anticancer drugs like Dox. Nucleolin receptors are overexpressed on tumor cells and can be used to selectively target these cells for the delivery of anticancer ligands and nanoparticles.[135] Incorporation of anti-nucleolin binding aptamers as hydrogel cross-linkers induced dissolution of the hydrogel upon nucleolin binding, thus effecting drug release in biological environments.[123]

In addition to small molecules as drugs, other therapeutically active molecules may offer new avenues to treat diseases. In this context, protein therapeutics are of emerging interest for many indications as they combine biological activity, target selectivity, biodegradability, and biocompatibility.[136] However, their low plasma half-life, blood circulation, and membrane penetration often limit in vivo usage.[137] Therefore, the exploration of novel delivery routes of protein therapeutics is of great interest and hydrogels represent an attractive option for generating local depots in vivo.
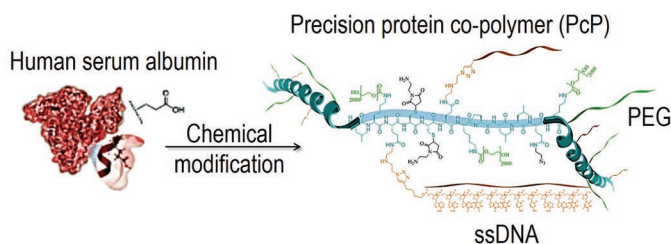
Osteoporosis represents a low bone mass disease and is characterized by a deterioration of bone tissue that is connected with a high occurrence of bone fractures.[138] Incident rates of osteoporosis increase with age and currently available treatments are mainly systemic, which can lead to severe side effects.[138] It has been shown that Rho-inhibiting C3 toxins selectively target bone-resorbing osteoclast cells which cause brittle bones

and enhanced risk of bone fractures.[139] Local delivery and release of the C3 protein toxin from a polypeptide-DNA hybrid hydrogel has been demonstrated as a promising method for the spatial and selective modulation of these bone-degrading osteoclast cells (**Figure 7**).[49] The hydrogel consisted of two components: a) the polypeptide backbone derived from human serum albumin with grafted ssDNA sequences and b) dendritic DNA providing four sticky ends, two of which are complementary to the grafted ssDNA sequences for cross-linking and the other two hybridize the DNA-tagged C3 proteins.[47–49] The resulting DNA hybrid hydrogel revealed self-healing properties allowing local positioning by injection and controlled degradation by proteases and nucleases that boosted C3 release. It effectively reduced osteoclast migration and resorption activity in vitro without affecting the activity, viability, and proliferation of bone-forming osteoblasts. Thereby, the hydrogel offers great potential for the local improvement of bone quality in osteoporotic bone and patient-specific prophylactic treatment of bones with a high fracture risk is conceivable.[49]
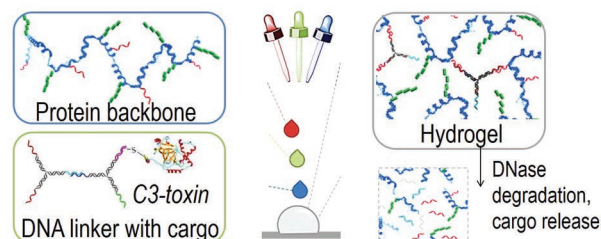
Another approach is based on aptamer-containing hydrogels that directly capture the bioactive molecules so that no chemical modifications of these molecules are required. The aptamer-containing hydrogels are particularly useful for the delivery and sustained drug release to specific cells or tissues. They are capable of storing and releasing bioactive molecules either sustainably,[70,71,123] periodically,[72] or through external,[73,74,122,140] or mechanical triggers via direct cell–material interactions such as traction forces[141] by introducing aptamers that specifically bind the active ingredients. When incorporated into gels, aptamers specific to signaling proteins such as anti-platelet derived growth factor-$\beta\beta$ (PDGF-$\beta\beta$)[70–74] or vascular endothelial growth factor (VEGF)[73] increase the local concentration of these proteins thus retaining them longer in the gels compared to nonfunctionalized hydrogels. A triggered and sequential release of these proteins was achieved by applying the respective DNA replacement strands and subsequent hybridization and strand displacement as depicted schematically in **Figure 8**a.[72–74,122,140]

The principle of triggered and sequential protein release from DNA hydrogels was further expanded by spatially controlling the immobilization of proteins. Therefore, Zhang et al. proposed a simplified approach using UV-light-induced thiol-ene click chemistry to produce an aptamer-patterned poly(ethylene glycol) (PEG) hydrogel by applying a photomask (Figure 8b,c). The photomask allows patterning within the hydrogel, where protein-binding aptamers are conjugated to the polymer network in the areas exposed to UV light.[142,143] Protein loading occurs only within these regions and its release is triggered by hybridization of displacement strands to the aptamers (Figure 8b).[142] Using different protein-binding aptamers, multiple distinct proteins could be captured and released in a controlled fashion. It has been demonstrated that after incubation in a mixture of VEGF and PDGF-$\beta\beta$ proteins the respective binding and retention only occurred in those regions of the gel that were functionalized with the corresponding aptamer sequences (Figure 8c).[143] Using the displacement strand strategy, the proteins were released at predetermined time points. This combination of photo-patterning with two different aptamers gave both spatial and temporal control over the
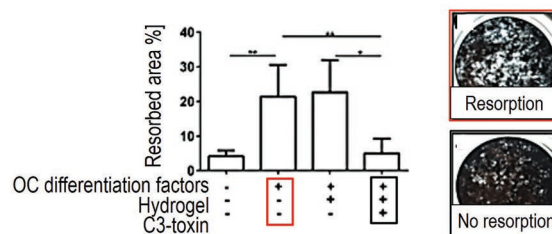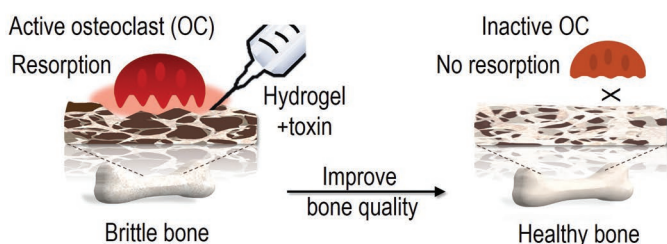
Figure 7. DNA-based hydrogels for application in bone regeneration. a) The DNA-protein hybrid hydrogel is assembled by cross-linking a serum albumin–derived polypeptide copolymer with grafted PEG and ssDNA side chains with dendritic DNA origami cross-linkers. Controlled release occurs via DNases. b) Design of injectable hydrogels that could inhibit osteoclast formation for local improvement of bone quality. Controlled release of C3 hydrogels effectively reduced osteoclast resorption activity without affecting osteoblast, bone-forming cells, in vitro. Adapted under the terms of the Creative Commons Attribution-NonCommercial 4.0 International license.[49] Copyright 2017, The Authors.

release of two separate proteins even in complex environments such as human serum.[143] Good cytocompatibility was demonstrated suggesting promising potential for in vivo usage. Therefore, protein delivery is combined with patterning to realize spatially controlled immobilization and release of different proteins simultaneously or sequentially at distinct times and concentrations within 3D hydrogels.[143] The approach offers great potential to construct smart protein carriers for controlled release or biomimetic 3D micro-environments for cells.[142,143]

However, for certain applications it is not feasible to use DNA strands or other small molecules as the release trigger. Consequently, external triggers, such as light of specific wavelengths have been investigated to stimulate drug release. Light-induced protein release from DNA hydrogels via sequential photoreaction and hybridization in a self-programming fashion is particularly attractive as light represents a well-controllable external stimulus.[78] An external light signal induces breakage of light-responsive groups within the DNA hydrogel releasing an oligonucleotide as an internal molecular signal.[78] These oligonucleotides diffuse to neighboring DNA–protein complexes replacing the DNA, dissociating the DNA–protein complex and thus releasing the protein cargo. Therefore, controlled PDGF-$\beta\beta$ protein release was demonstrated in a wound closure assay and the potential to regulate smooth muscle cell migration by the signal was highlighted.[78]

In addition to the discussed systems, the Almquist group has recently published a new approach that goes beyond the current usage of external triggers.[141] They eliminated the need for passive activation by, e.g., hydrolysis, spatially diffusive triggers such as enzymes and pH changes, and even sophisticated exogenous triggers such as light by implementing cellular
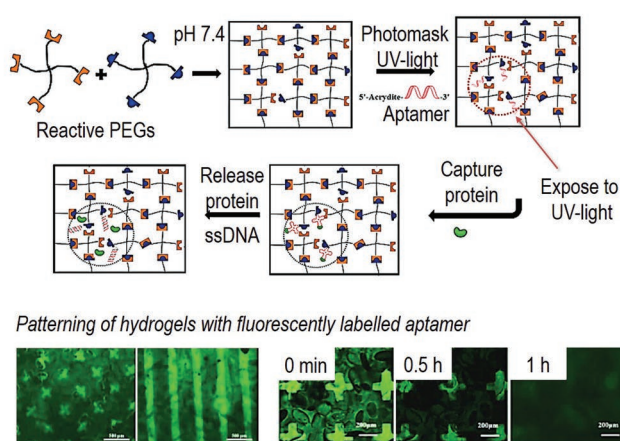
traction forces as innate stimulus. Briefly, aptamers were used as a substrate-independent, programmable platform, which is responsive to cellular traction forces, so-called TrAPs (traction force-activated payloads). Making use of the fact that aptamer binding constants rely on their ability to fold into a structure that binds to the relevant pocket on the targeted protein, Stejskalovà et al. developed a system where the activation of bioactive proteins was actively triggered by dynamic interactions of cells with the biomaterial.[141] Here, the exertion of cellular traction forces triggered unfolding of the aptamers, which drastically reduced their binding affinity thus releasing the bound protein payloads, i.e., PDGF-$\beta\beta$ or VEGF-165. These aptamers were conjugated to a cell-adhesive peptide on the one end, and a chemical group on the other end enabling their conjugation to the polymer scaffold of interest. The functionality of such dynamic biomaterials was verified on planar structures and within clinically relevant 3D collagen sponges.[141] Even though the authors did not report on biomedical applications, based on the presented advanced proof-of-principle in vitro data, they suggest that this strategy forms the basis of a new method for integrating latent growth factor signaling within a wide range of biomaterial-based systems that are activated by their direct dynamic interactions with cells, opening new possibilities in designing dynamic biomaterial systems.[141]

The advantages of DNA-containing hydrogels for drug delivery applications rest on their programmability, reproducible drug-loading, biocompatibility, and biodegradability. In the context of cancer targeting, the possibilities offered by DNA hydrogels to release one or more drugs at precise locations and in predetermined doses may help overcome the resistance of certain hard-to-treat tumors, while minimizing detrimental

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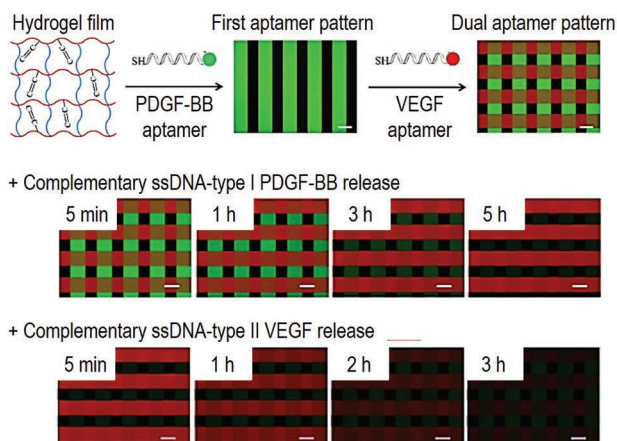MATERIALS**
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Figure 8. a) Triggered cargo release from DNA-based hydrogels for therapeutic approaches. Release of multiple protein-drugs via strand displacement. Aptamer-functionalized hydrogels are designed with adjustable release rates at predetermined time points using complementary sequences as biomolecular triggers. The color intensity of the balls indicates the protein amount, whereas light colors indicate fewer bound proteins. Adapted with permission.[73] Copyright 2012, American Chemical Society. b) Patterning and controlled release of therapeutic proteins as smart DNA-based hydrogels in biomedicine. 3D bioactive patterned hydrogels as biomimetic environments with spatio-temporally controlled release of bioactive proteins. The selective protein capturing and their spatial distribution were realized in an aptamer-patterned hydrogel. The system is based on the specific affinity between the aptamer and protein. Addition of complementary DNA strands (ssDNA) of the aptamer dissociates the protein cargo from the aptamer at desired time points and concentrations based on DNA hybridization. In this example, the spatial release of the protein thrombin in an aptamer-functionalized PEG hydrogel is shown within 1 h by adding the respective ssDNA. Adapted with permission.[142] Copyright 2017, Royal Society of Chemistry. c) Patterned hydrogel for spatio-temporally programmable capture and release of multiple proteins. To control the capture and release of multiple proteins in different hydrogel regions and at desired time periods was realized in aptamer-patterned hydrogels. By conjugating DNA of different aptamers via photoclick chemistry, spatio-temporally adjustable release rates can be realized for multiple proteins as both aptamer–protein interaction and aptamer–complementary ssDNA hybridization are highly specific. Here, two different aptamer–protein dissociations were triggered through complementary ssDNA releasing growth factors from aptamer-patterned hydrogel films providing a platform for aptamer functionalization and controlled protein release from hydrogel films. Adapted with permission.[143] Copyright 2018, American Chemical Society.

effects to healthy cells. However, the safe positioning of such local depots needs to be considered and they may not be suitable for addressing distant metastasis. As more stimuli for releasing compounds from DNA hydrogels are being developed, the potential uses also increase. Patterned hydrogel depots could be imagined as smart implants that can sense and react to biochemical processes of the individual patient, and release appropriate drugs in response to certain levels of analytes or metabolites. This would open great opportunities in advanced and personalized medicine.

3.3. Immunomodulatory DNA Hydrogels

The immune system represents the primary defense that protects the body against various infections and pathogens. Many vaccines have been developed to train the immune system to recognize pathogens.[144] Prophylactic vaccines are one of the most efficient therapies to prevent infective diseases, and they have been recognized for the treatment of certain cancers, namely, those of viral origin, such as hepatitis B virus and human papillomavirus (HPV).[144–147] However, many diseases are also associated with disorders of the immune system itself. On the one hand, an overactive immune system can be harmful to the person, e.g., in allergies, where foreign but harmless substances such as pollen, induce a severe immune reaction. In addition, healthy tissue can suddenly be mistaken as foreign and dangerous, resulting in a severe attack in autoimmune diseases. Diabetes Type I, rheumatic arthritis, multiple sclerosis, and Lupus fall under the latter category.[144,145] In such states of hyperactive immune responses during autoimmune and inflammatory diseases,[145] as well as transplant rejection,[145]

the immune cell activation has to be reduced.[125,144] On the other hand, hypoactive conditions that appear in certain cancers or chronic infections often require the opposite approach of increasing the immune cell activation.[125,144]

In previous years, many new small molecules, proteins, or polymers have been developed as immunomodulators, some of which were also integrated into polymeric platforms.[144] Hereby, conventional treatment approaches aimed at therapeutic immune system modulation by administration of soluble components, orally, nasally, or via local or systemic injection, and their dispersion throughout the body. However, only recently, polymeric scaffold-based immunomodulatory systems that can be implanted beneath the skin were reported and promise to treat different types of cancer, chronic infections, and autoimmune diseases.[144] In this context, the design of immunomodulating hydrogels has become a rapidly growing field of research as they could be designed at multiple length scales (macro- to nanoscale) to target organs (e.g., lymph nodes, lymphoid residing cells, and their intracellular compartments), function as surrogate lymphoid-like tissues, and deliver multiple biomolecules that could modulate the humoral and cellular immune response.[144] Here, DNA hydrogels are particularly interesting as they allow for the sustained release of bioactive molecules and guarantee intrinsic biodegradability over longer time frames.

Most research in the field of immunomodulatory DNA hydrogels centers on the utilization of unmethylated cytosine phosphate guanine (CpG) dinucleotide, the so-called CpG motif, in order to develop immunostimulatory soft materials for sustained release of signals.[148–152] The CpG motif is a well-known pathogen-associated molecular pattern that resembles a danger signal, and it is recognized by the mammalian immune system via toll-like receptor 9 (TLR-9).[148,153–156] Subsequent release of proinflammatory cytokines stimulates the immune system.[155,156] While the effects of CpG motifs have yet to be fully elucidated, it is clear that CpG rapidly activates a variety of cell types spanning B cells, natural killer cells, and antigen-presenting cells, including monocytes, macrophages, and dendritic cells.[155,156] The utilization of the CpG motif as a vaccine adjuvant seems promising and its implementation has been reported in a variety of DNA-based hydrogels, for applications spanning from immunotherapy against allergies to vaccination against cancer.[149,155–157] Besides, immobilization of CpG motifs within such gels can easily be obtained by hybridization. While the potent immune activating effect of CpG oligonucleotides was described the first time by Krieg et al. in 1995,[158] pointing out possible new applications as adjuvants, the first examples of DNA-based hydrogels containing the CpG motif[153,154] were reported by the Nishikawa group in 2010.[148] Building on this, newer systems with, sustained release of antigens for immunotherapy of, i.e., allergic rhinitis were subsequently described by the same group.[149–152] In a frequently reported strategy, six oligonucleotides and CpG motifs were assembled into dendritic DNA structures (**Figure 9**a,b).[149–152,159] The self-gelling and injectable CpG DNA hydrogel showed a high immunological potency and low toxicity as a vaccine adjuvant when loaded with ovalbumin (OVA), which is frequently used as a model antigen and serves as a proof-of-concept for many biomaterial-based vaccines[144] that is injected intradermally.[159] This was highlighted by the decrease in adverse reactions of the
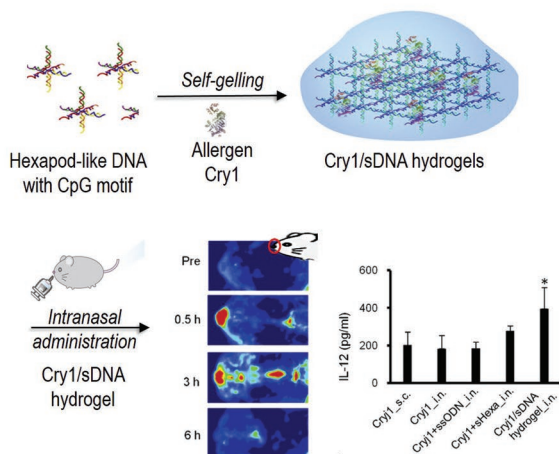
OVA/CpG DNA hydrogel compared to OVA injected with complete Freund's adjuvant or alum, which are commonly used in experimental (Freund's adjuvant) or clinical (alum) vaccine formulations.[159] Due to the self-gelling characteristics of the CpG DNA hydrogels, its administration into cavities or as a spray even allowed intranasal vaccination (Figure 9a).[149]

Rhinitis is caused by ragweed pollen and subcutaneous injection of CpG DNA conjugated antigen effectively prevented this disease in clinical studies.[160,161] The intranasal vaccination of Japanese cedar pollen (JCP) in mice also induced immunotherapeutic effects against allergic rhinitis.[149] CpG hydrogels loaded with Cryj1, a major JCP allergen, effectively induced allergen-specific immune responses in mice after intranasal administration, which was partially due to the sustained release of this antigen from the hydrogel.[149] The authors also proposed DNA hydrogels as a mucosal delivery system for shorter immunotherapy with a lower burden regarding their application by avoiding repeated subcutaneous injections.[149] To further broaden the applicability of the gel for oral delivery of CpG DNA antigens, the development of DNA hydrogel microspheres that were coated with chitosan was proposed to improve the stability and increase the intestinal transit time.[150] Further modifications focused on increasing the duration of sustained antigen and CpG DNA release from the hydrogels to increase their potency.[151,152] Two approaches for modifying the gels have been tested, namely, making use of electrostatic interactions of the cationic polymer chitosan and DNA for stabilization as the first approach and hydrophobic interactions from additional cholesterol groups as the second approach.[151,152] As a result, more efficient specific immune responses of OVA-loaded CpG-DNA hydrogels were reported compared to the nonstabilized hydrogels.[151,152]
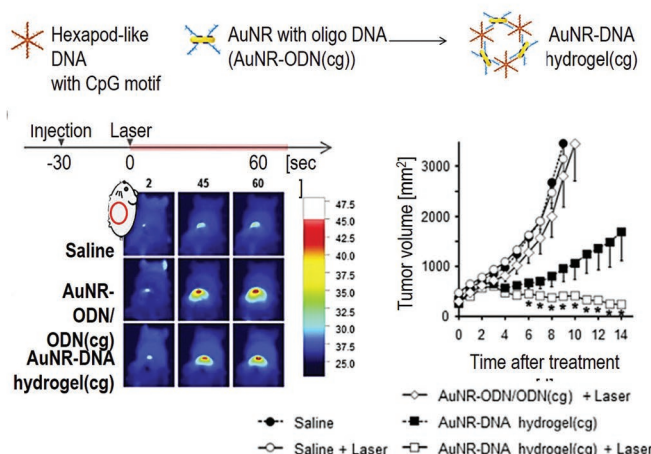
While the activation of the immune system for positive therapeutic effects has been a central but still elusive challenge in immunology and oncology for decades, recent clinical trials have significantly increased the chances of survival of patients with metastatic melanoma, for which conventional therapies have failed.[147] In this context, the emergence of targeted therapies and the ever advancing understanding of how antitumor immune responses are regulated, suggest that active immunotherapy is ideal to achieve efficient treatment of cancer patients.[147] Hereby, it is pivotal to break the immunotolerance of the tumor and induce robust immunoresponses of the patient by applying a suitable vaccine and/or immunostimulant.[162]

Umeki et al. further advanced the introduced immunostimulatory CpG DNA hydrogels for cancer immunotherapy, enhancing the antigen-specific antitumor immunity of the host by encapsulation of immune cells within the hydrogel, thereby, achieving co-delivery of the antigen and immune cells.[157] The hexapod-like DNA nanostructures featuring CpG motifs were also utilized in combination with gold nanoparticles to achieve tumor photothermal immunotherapy (Figure 9b). Here, Yata et al. created a composite-type DNA hydrogel, where gold nanorods functionalized with oligomers were intended to hybridize with immunostimulatory dendritic DNA during hydrogel formation.[163] These hydrogels, upon laser irradiation, release the hexapod-like DNA which efficiently stimulated immune cells.[163] Simultaneously, laser
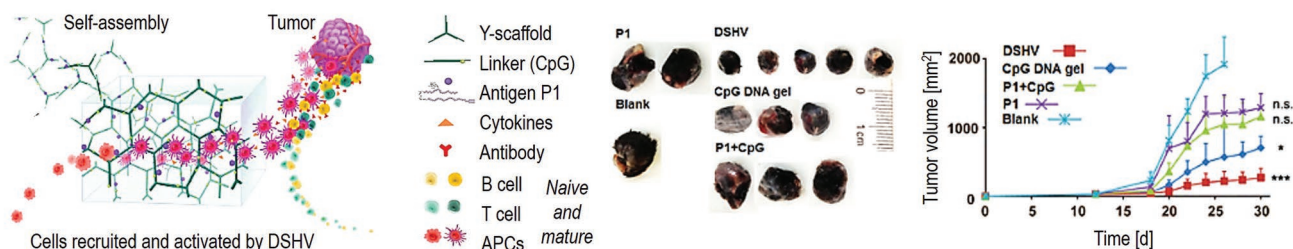
Figure 9. Immunomodulatory DNA hydrogels. a) Nasal vaccination by application of the controlled release of allergens using self-gelling immunostimulatory DNA for effectively inducing immune responses. Immunostimulatory DNA hydrogels are obtained by mixing hexapod-like DNA nanostructures using six oligodeoxynucleotides each, including an unmethylated cytosine–phosphate–guanine sequence (CpG motif), with the allergen (Cryj1). The allergen features slow release from the stDNA hydrogel, which is retained in the nasal cavity after intranasal administration. Intranasal immunization of mice with the Cryj1/sDNA hydrogel results in high levels of Cryj1-specific IL-12. Adapted with permission.[149] Copyright 2015, Elsevier. b) Tumor photothermal immunotherapy using DNA-based composite hydrogels. Applying a composite-type immunostimulatory DNA hydrogel consisting of hexapod-like DNA nanostructures with CpG sequences and gold nanoparticles allows for the efficient stimulation of immune cells upon laser irradiation of the hydrogel due to the release of hexapod-like DNA nanostructures. The intratumoral injection was followed by laser irradiation which increased the local temperature. Significantly retarded tumor growth has been demonstrated. Adapted with permission.[163] Copyright 2017, Elsevier. c) Designable immune vaccine systems based on DNA hydrogels. An injectable DNA supramolecular hydrogel vaccine (DSHV) system was designed to efficiently recruit and activate APCs in vitro and in vivo, thereby, mimicking the function of a lymph node where the APCs are recruited and activated by the high local concentration of CpG. After injection, naive APCs were recruited and activated by the DSHV system, resulting in subsequent interaction of mature APCs with other immune cells yielding strong immune responses and antitumor effects. The DSHV can be fabricated through the self-assembly of DNA-based Y-scaffold and linker, as well as antigen P1. CpG sequences are included in linker sequences, which are marked in bright green color. The antigen P1 contained a B-cell and a T-helper cell epitope is shown as a purple ball. Adapted with permission.[162] Copyright 2018, American Chemical Society.

irradiation at 780 nm increased the local temperature, which consequently enhanced tumor-associated antigen-specific IgG levels in serum and interferon production from splenocytes, white blood cells from the spleen.[163–165] Such treatment significantly retarded tumor growth and extended the survival of the tumor-bearing mice.[163]

In contrast to the presented hydrogels that were designed to present and deliver certain antigens, recently, Shao et al. developed an injectable DNA hydrogel vaccine (DSHV) that behaves like an endogenous lymph node (Figure 9c).[162] After intraperitoneal or subcutaneous injection, the DSHV served as an artificial lymph node, where the antigen-presenting cells (APCs) were recruited and activated in vitro and in vivo by high local CpG concentrations resulting in strong immune responses and antitumor effects. The recruiting of APCs into the gel and their subsequent activation effected by high antigen concentrations sets this hydrogel system apart from the previously presented vaccine-releasing systems.[162] These findings underline the emerging relevance of supramolecular DNA-based hydrogels as a general platform for tumor vaccination and cancer therapy in the future.[162] Complementing the field of immunostimulatory DNA hydrogels, recent attempts also envision the delivery of immunoinhibitory nucleic acids for the treatment of autoimmune diseases, e.g., systemic lupus erythematosus, related to enhanced TLR9-mediated signaling.[156,166] The design of immunomodulatory materials is still in its infancy. With the advent of tumor immune therapy, the application of soft DNA hydrogels for the local, controlled release of immunogenic agents promises new therapeutic options for the treatment of certain cancers, infections, and other diseases.

3.4. DNA Hydrogels for Cell-Based Approaches and Tissue Engineering

The following section deals with DNA hydrogels that interact specifically with cells. These interactions are applied toward tissue engineering where extracellular matrix (ECM) mimics are generated that can precisely position bio-signals and even cells themselves, e.g., in tissue cultivation, as well as capture specific cells from circulation.

Tissue engineering has emerged as a key technology in regenerative medicine.[167–169] The introduction of cells and tissue constructs into the human body to replace or repair patient tissue is still a challenging endeavor as the "engineered" tissue should replicate the structure and function of the normal, healthy tissues as closely as possible.[170,171] Hydrogels have long since been applied as soft matrices due to their similarities to soft tissue[171] and their innate structural and compositional similarities to the ECM provide an adequate framework for cellular proliferation and survival.[172,173] As a result, their usage as ECM mimics is a prevalent choice and different kinds of hydrogels with diverse chemical and physical properties have been proposed to enable cellular attachment and differentiation in biodegradable matrices and facilitate solute nutrient transport.[171,174]

The thixotropic nature inherent to many DNA hydrogels is particularly useful toward tissue engineering applications, as new processing techniques could be applied such as bioprinting to mold the hydrogels, as bioink, into creating sophisticated 3D shapes[26] like 3D multilayers with complex structures and cell patterns.[26] Such soft materials could be preformed to fit various cavities when administered into the patient.[175] In addition, DNA-based hydrogels allow for the programmed dynamic and reversible display of various signals, positioned with nanoscale accuracy, to facilitate and control interactions with the ECM or cellular membrane.[9,176] The presentation of bioactive signals such as the RGD peptide motif that binds to integrin cell surface receptors[9] is essential when designing ECM mimics for tissue engineering.[9,171] DNA-based hydrogels with short bioactive peptide sequences such as RGD, IKVAV, or growth factor-derived sequences were conjugated to DNA strands, which then hybridized with sticky ends of surface-exposed DNA.[9] Richards et al. developed a DNA-based system for surface modification of, e.g., acrylamide hydrogels yielding polyvalent display of affinity ligands for cell culturing (**Figure 10**a).[177] The synthesis of a polymeric DNA scaffold via hybridization chain reaction starting from single-stranded oligonucleotides was followed by hybridization of aptamers onto the hydrogel surfaces. As each DNA scaffold provided multiple sites for aptamer hybridization, surface functionalization with polyvalent aptamers was realized in a straightforward manner. Consequently, hydrogel surfaces functionalized with polyvalent ligands enhanced ligand display and 44% more cell binding was observed after 90 min compared to surfaces directly conjugated with equal amounts of cell-specific aptamer.[177] This approach is especially interesting when limited surface areas or insufficient surface reaction sites are available.[177]
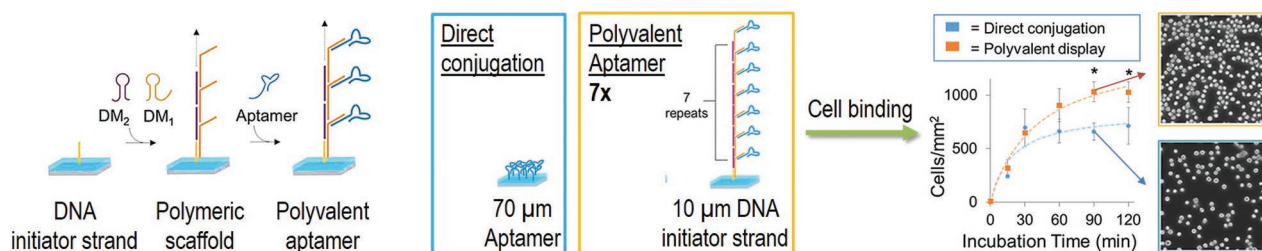
This study demonstrated a novel and highly efficient DNA-based approach for the functionalization of surfaces employing polymerization of affinity ligands. However, the importance of multivalency for cell regulation and the control over dynamic signal display is another desirable material characteristic. Freeman et al. described a thin alginate layer that employed DNA to dynamically control and spatially arrange multiple bio-active signals in order to mimic features of the ECM.[9] Thereby, bioactive peptide-DNA hybrid strands were immobilized on the surface through complementary DNA tethers, so-called surface strands (Figure 10b). A series of different DNA tethers varying in the degree of base-pair complementarity to the surface strands allows for programmed displacement of the functional DNA strands resulting in reversible switching of bioactivity. Thus, orthogonal and reversible display of multiple biomimetic signals as well as synergies between bioactive epitopes, which are spatially dependent, were promoted on a biomaterial using DNA as an adjustable spacer. As a result, cells were instructed to migrate or regroup as a direct response to a dynamic switch of exogenous signals for proliferation, such as a mimic of the fibroblast growth factor 2, or differentiation, like the peptide IKVAV.[9] Similarly, this technique could be beneficial for tissue engineering applications when used to modify hydrogel surfaces to direct cellular responses.

In addition to functional signal display, mechanical cues arising from ECMs also affect cellular properties and, therefore, the mechanical and rheological properties are of high significance in biomaterial design. Jiang et al. demonstrated that the tunable mechanical properties of polyacrylamide gels cross-linked with acrydite-oligonucleotides affect the neurite outgrowth of spinal cord neuronal cells in vitro.[44] By varying the length of the cross-linking strands or the stoichiometry of the linker strand to the bound oligonucleotides, they demonstrated gel stiffnesses ranging from 100 Pa to 30 kPa. Such materials could be important for neuronal regeneration, e.g., in spinal cord injury as well as for neural tissue engineering including neuropathological conditions and neurodegenerative diseases where the mechanical stiffness of tissue is being altered.[44,178,179]

However, despite the advances in the self-assembly of artificial hierarchical systems, approaches to create reversible and tunable structures that can be manipulated across different length scales remain challenging. Recently, Freeman et al. reported fibrous DNA hybrid hydrogels featuring reversible and dynamic self-assembly of superstructured networks mimicking the ECM (**Figure 11**a). Thereby, nanofibers were formed from peptide amphiphiles as well as peptides containing a covalently linked oligonucleotide terminal segment and were reversibly cross-linked by Watson–Crick base pairing between complementary oligonucleotides on separate fibers. Importantly, the resulting hydrogels exhibited hierarchical structures, which arose from the DNA-peptide amphiphiles' dynamic reorganization. In contrast, disassembly occurred in response to chemical triggers such as ssDNA that mediated strand displacement.[180] Two populations within the gel were observed, twisted fiber bundles as well as single fibers, and the switching between these two morphologies induced changes in cell activity, which was demonstrated for astrocytes (Figure 11A). A reactive phenotype was only observed when the astrocytes were cultured on the bundled fiber hydrogel resulting in the upregulation of cell marker proteins like glial fibrillary acid protein (GFAP) and an increase in reactive oxygen species (ROS).

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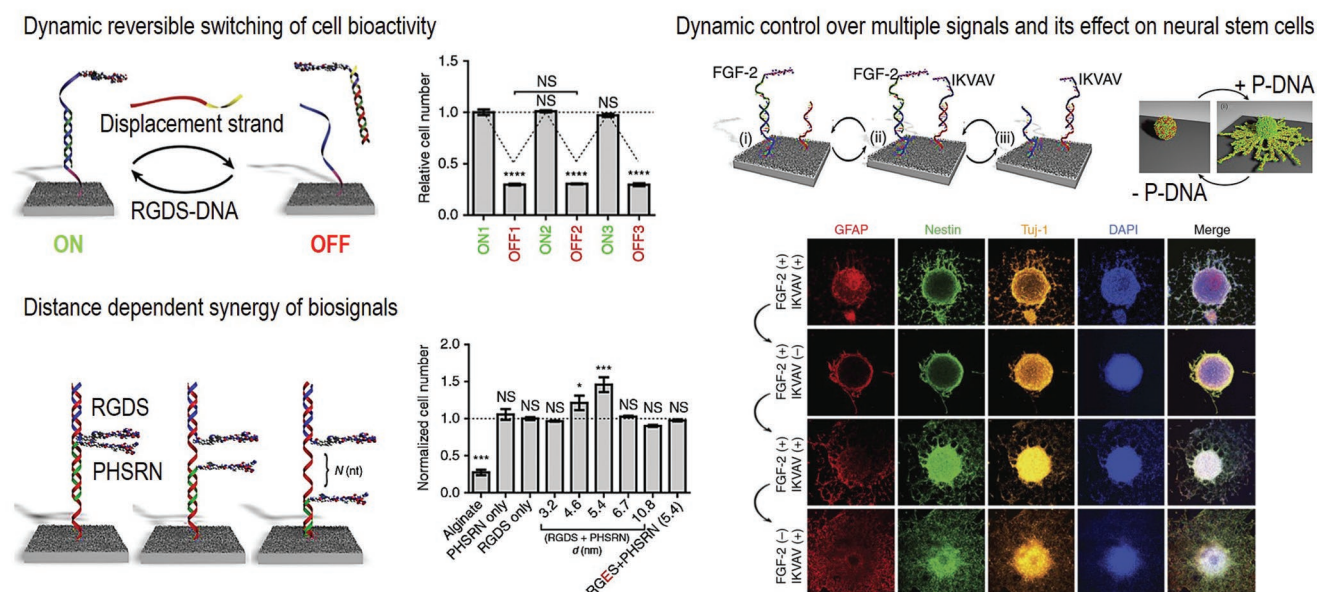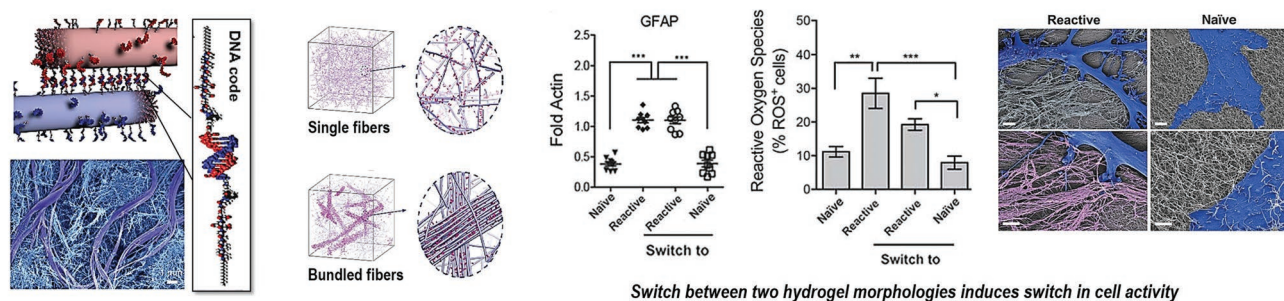**MATERIALS**
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Figure 10. a) Schematic illustration of DNA polymerization for enhanced display of aptamers and comparison of surfaces with equal aptamer amounts. At direct conjugation, individual aptamers were directly conjugated to the hydrogel while polyvalent display features polyvalent aptamers formed on the hydrogel surface. The kinetic analysis of cell binding on surfaces with the aptamer amount of 7 × and the comparison of cell binding on surfaces with equal amounts of aptamer. Adapted with permission.[177] Copyright 2014, American Chemical Society. b) Dynamic reversible switching of cell bioactivity via toehold-mediated strand displacement. The bioactive strand is removed by a fully complementary displacement strand, thus switching bioactivity OFF; this process regenerates the surface strand, allowing for a second addition of the RGDS-DNA to turn bioactivity back ON. This process can be repeated for multiple cycles. DNA surface strands with variable spacer regions tune the distance between the peptide signals RGDS and PHSRN. The right panel shows immunofluorescence images of neurospheres subjected to dynamic and orthogonal presentation of IKVAV and FGF-2 signals. Adapted under the terms of the Creative Commons Attribution 4.0 International license.[9] Copyright 2017, The Authors.

As these important phenotype transformations in astrocytes that are linked to brain and spinal cord injury as well as neurological diseases were demonstrated to be modulated by architectural cues, the authors suggest that future therapeutic strategies that "defibrillate" glial scars could be explored to reverse neural pathologies through astrocytic fate decisions.[180] In a follow-up work, Daly et al. further demonstrated the powerful concept of combining DNA with peptides.[181] Compared to the work of Freeman et al., where peptide amphiphiles were used,[180] here, the peptide–DNA hybrids were created with the very short dipeptide Fmoc-FF–OH[181] that is known to assemble efficiently into nanoscale fibers. The combination with oligonucleotides of different lengths encoded the formation of reversible supramolecular hierarchical fibrous assemblies that were

tunable by temperature or pH changes, as well as the addition of soluble triggers such as DNase. Variation in the length of the DNA segment had an impact on the morphology, dimensions, and helicity of the obtained structures. The presented data suggests that this technique could serve as a useful handle for the design of tunable, self-assembled architectures, and the authors envision its application in the areas of tissue engineering, drug delivery, and sensing.[181]

The precise positioning of a large number of specialized cells with different functions within a material is another central challenge in tissue engineering.[182] Additionally, in multicellular organisms, important cellular processes such as proliferation and differentiation are regulated by signaling from the ECM. A material that is able to recognize particular
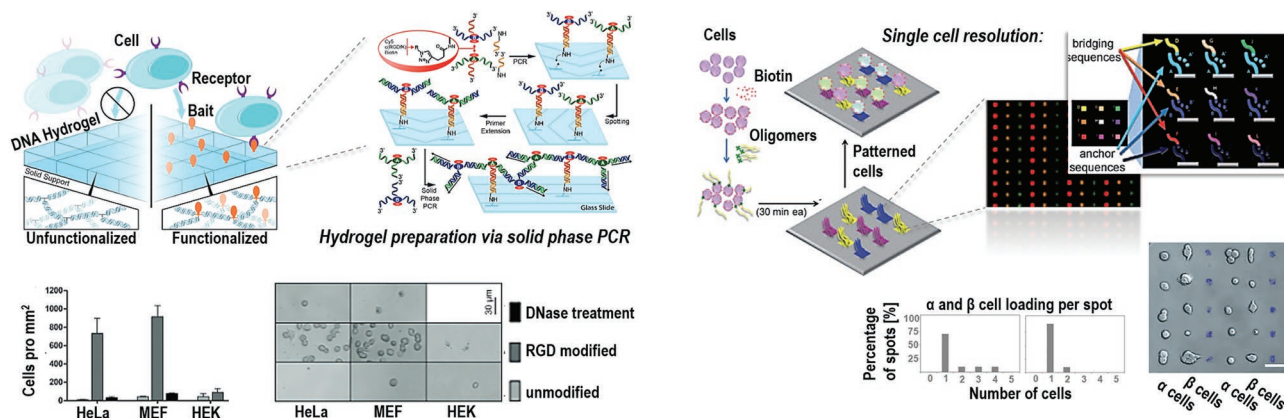
Figure 11. DNA-based hydrogels in tissue engineering—mimicking tissue. a) Reversible and dynamic self-assembly of superstructured networks in DNA hybrid hydrogels mimicking the ECM. Applying DNA hybridization for further cross-linking of peptide amphiphile fibers yields hydrogels with hierarchical structures due to the DNA–peptide amphiphiles' dynamic reorganization. Disassembly occurs in response to chemical triggers. Two populations can be observed within the gel, consisting of twisted fiber bundles as well as single fibers, and the switching between these two morphologies can induce a switch in cell activity which is shown for astrocytes. A reactive phenotype (cells are false colored in blue) was observed when cultured on bundled fiber hydrogel (false colored in pink) resulting in the upregulation of GFAP and an increase in ROS. Adapted with permission.[180] Copyright 2018, American Association for the Advancement of Science. b) Designer ECM based on DNA–peptide networks with cell adhesion properties. The networks are constructed using covalently branched DNA as primers in PCR. As a covalent coating of surfaces, this DNA-based material shows cell-repellent properties in its unfunctionalized state. Adhesiveness toward specific target cells is introduced with the respective biological signal peptide upon application of functionalized primers. DNase treatment enables the release of the adhered and viable cells under mild conditions. Adapted with permission[183] Copyright 2016, Wiley-VCH. c) Multiplexed patterning of cells at single-cell resolution. In this approach, the cell membrane proteins are biotinylated and then addressed via streptavidin-conjugated oligonucleotides. Patterning of the respective DNA spots in a microarray and building an islet construct that includes, e.g., mouse α- and β-islet cell lines, represents the construction of model pancreatic tissues as an avenue toward engineering islets for type 1 diabetics. The transfer of patterned cells into thin, stackable hydrogel films extends the pattern into three dimensions. Other than tissue engineering, cell-based assays or fundamental studies of cell–cell interactions are potential fields of application. Adapted with permission.[187] Copyright 2011, Wiley-VCH.

cell types and then allows for the controlled attachment of these cells in a programmable way would be highly desirable.[183] One approach in this direction focused on creating DNA-network-based functional hydrogels as an artificial designer ECM on solid support.[183] The networks were generated by enzymatic elongation via PCR. In a first step, amine-functionalized primers and two different branched primers were used for PCR while the resulting product became conjugated to an activated glass surface. In a next step, primers that did not participate in the first PCR were elongated in a primer-extension reaction and the usage of branched primers leads to their incorporation into the surface bound network. Here, the utilization of functionalized primers, i.e., modified with the cyclic peptide c(RGDfK), a bait molecule that is known to enhance cell

adhesion of endothelial cells and fibroblasts gave rise to selective cell attachment through specific interactions with cell-surface markers (Figure 11b). In this way, cell-repellent properties of the unfunctionalized DNA-based hydrogel were converted to adhesiveness toward specific target cells when functionalized with the respective bait peptide. The specific response of cells to the DNA-modified surfaces was demonstrated by applying different cell types like HeLa or mouse embryonic fibroblasts that are known to bind the RGD peptide motif and cells such as HEK293T cells that were reported to not interact.[184–186] Notably, the nature and concentration of displayed molecules can be controlled to address different types of cells while DNase treatment allows for mild and efficient detachment of the adhered and viable cells.[183]

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The programmability of DNA hydrogels regarding their composition and structure is an essential feature, as engineered tissue constructs require correct spatial positioning of cell types to mimic the anatomical microarchitecture in native tissue.[187] Therefore, the ability to control the positioning of cells within a material down to individual cells would offer great opportunities for constructing complex tissues with distinct hierarchies. Oligonucleotide-modified hydrogel surfaces selectively recognize distinct cell types with single cell-resolution due to complementary binding. For example, Vermesh et al. reported multiplexed patterning of cells at single-cell resolution by labeling different cell types with a unique complementary oligonucleotide strand in order to covert DNA arrays to defined cell arrays. The subsequent transfer of the patterned cells into thin, stackable hydrogel films allowed the assembly of 3D islet tissue-like constructs (Figure 11c).[187]
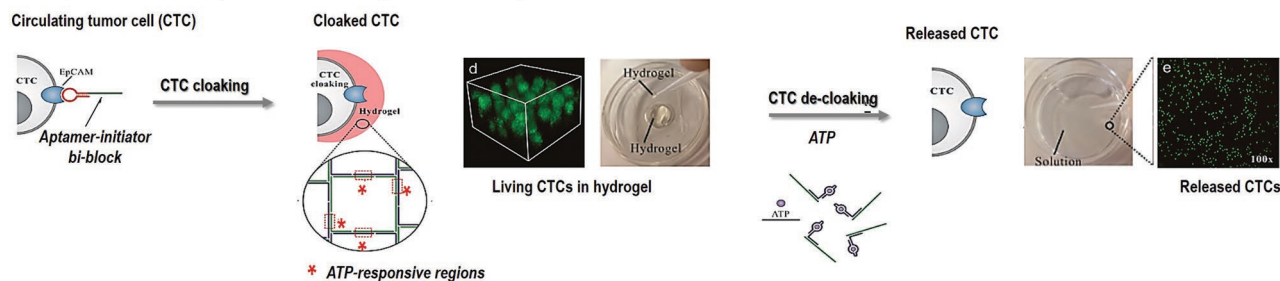
Apart from the applications in tissue engineering, controlled DNA hydrogel–cell interactions have been exploited for the specific binding of circulating tumor cells (CTCs) (**Figure 12**a). CTCs are viable tumor-derived cells that circulate in peripheral blood and contain molecular information about the primary tumor. Capture of such CTCs for their direct detection could provide a simple and effective approach for early cancer diagnosis. Selective isolation would allow for predictive diagnosis and therapy avoiding complex invasive tumor biopsy.[188–192] However, despite great promise, many CTC detection techniques are costly, time-consuming, or need advanced instrumentation.[69,192] Another critical issue that still needs to be addressed is the specificity of the "cell catch" step, where the current technology can only achieve 50% purity in the identified CTCs. This is insufficient for reliable real-time monitoring of the cancer therapy response, early cancer detection, or the identification of CTC subpopulations such as metastatic precursor cells.[189] Taking advantage of the unique features of DNA nanomaterials, several approaches to construct DNA-based hydrogels that provide the ability to capture live cells with high specificity and in real-time, as well as releasing the captured, vital cells in response to an external trigger, have been reported. In contrast to many other systems, which involve harsh capturing conditions inducing nonphysiological stress and often damage the CTCs, aptamer-functionalized nanostructures showed great promise for CTC capture.[75] Song et al. described a novel strategy for the highly specific cancer cell catch and gentle release. They designed an aptamer-triggered in situ gelation mechanism for liquid cloaking of CTCs for live-cell analysis. The aptamer-toehold conjugated DNA staple strands, aptamer-initiator diblocks, were chosen to bind specifically to epithelial cell adhesion molecule (EpCAM) on the cell surface that is overexpressed by most cancer cells and induces the clamped hybridization chain reaction (HCR). Notably, the cancer cell recognition elements are not limited to EpCAM but can be substituted by other proteins expressed on the cell surface. While many systems often also lack the ability for live cell release after capturing,[190] implementation of ATP-responsive regions in the clamped HCR yielded a stimuli-responsive DNA hydrogel which provides both simple and gentle CTC capturing and release, yet has great potential to gather reliable information on the primary tumor.[75]

Another system for cancer cell capturing on a hydrogel surface was described by Zhang et al. who employed aptamer-functionalized polyacrylamide hydrogels for triggered cell release via either complementary sequences or restriction endonucleases (Figure 12b). They described systems with low unspecific cell binding, but high specificity toward human leukemic lymphoblast cancer cells on the hydrogel surface and excellent release efficiencies while maintaining cell viability.[77,193] Aptamer-mediated cell recognition was also applied by Li et al. to develop a *Drosera*-bioinspired hydrogel for catching and killing cancer cells (Figure 12c). Mimicking the carnivorous plant *Drosera* which presents an adhesive surface to the environment and releases digestive enzymes on its leaves, a novel hydrogel capable of performing multiple functions, more precisely, a hydrogel with target-catching and drug-releasing functions was designed. To achieve this, a bifunctional hydrogel was generated by two hydrogel layers, both of which were functionalized with oligonucleotides. The top layer served as a surface for capturing target cells through nucleic acid aptamers. The bottom layer hydrogel was chemically functionalized with a double-stranded DNA, constituting a depot for sustained sequestration of small toxic drugs. In vitro studies with human leukemic lymphoblasts showed that the cancer cells can be caught efficiently by the hydrogel followed by Dox release killing the cells. Repetitive display of the cell-catching aptamer on the cell surface combined with the sustained drug release further allows for killing cancer cells in a continuous fashion.[76] These examples highlight the exciting opportunities in creating cell-compatible matrices from DNA hydrogels. The combination of adaptable mechanical properties, programmability, selectivity, and biocompatibility make DNA a promising material for various applications in tissue engineering.
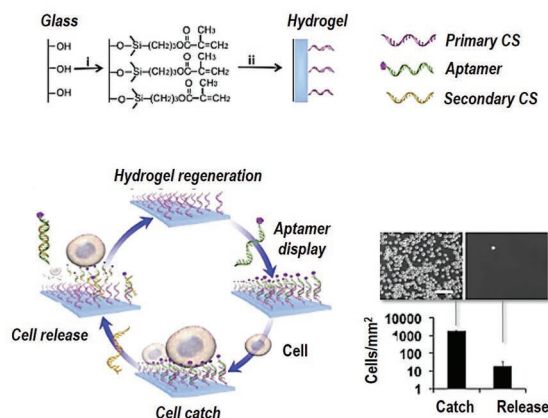
4. Conclusion

The recent progress in preparing DNA-based hydrogels has made this material class accessible for exciting applications in the biomedical field. DNA building blocks provide unique characteristics that have prompted the development of highly sensitive biosensors, drug delivery systems, and cellular scaffolds for regenerative therapies. We anticipate that these examples only signify the beginning of a much larger trend that is to follow in the future. For example, the aptamer technology enables the design of DNA gels that can detect almost any type of analyte with high selectivity and sensitivity. New aptamers are easy to generate, leading to an ever-growing number of addressable targets. The same approach is also useful in selectively triggering the release of drugs, where DNA-based systems can provide a biocompatible and often more selective alternative to conventional chemical linker strategies. New concepts, such as rechargeable drug reservoirs, can now be conceived due to the programmable and reversible interaction of complimentary DNA strands. Furthermore, the combination of novel DNA materials with new treatment approaches, as shown exemplarily for immunotherapy, holds great promise in the future for tackling diseases with challenging diagnoses, insufficient or nonexistent treatment options. Finally, interesting mechanical properties

a) Recognition, cloaking, and decloaking of circulating tumor cells

b) Controlled cell-type specific catch and release

c) Drosera-inspired cell attraction, catch and killing of diseased cells
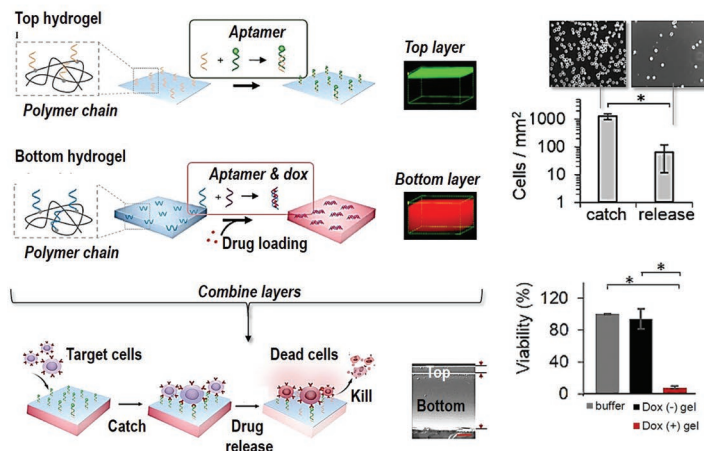
Figure 12. a) DNA-based hydrogels for cell-based applications in anticancer therapy. Recognition, cloaking, and de-cloaking of circulating tumor cells (CTCs) via DNA hydrogel with aptamer-toehold. Capturing and analyzing rare live CTCs allow for predictive cancer diagnosis as they contain molecular information on the primary tumor. The aptamer-triggered clamped hybridization chain reaction (atcHCR) can be applied for in situ identification and subsequent cloaking/decloaking of CTCs by porous DNA hydrogels. A DNA staple strand with aptamer-toehold biblocks specifically recognizes epithelial cell adhesion molecule on the CTC surface. Thereby, cells are captured directly with minimal cell damage. De-cloaking of CTCs using controlled and defined chemical stimuli can release living CTCs without damages for subsequent culture and live cell analysis. Adapted with permission.[75] Copyright 2017, American Chemical Society. b) DNA-based hydrogels for application as cell-type-specific catch and release platforms. Programmable hydrogels for controlled cell catch and release using aptamer hybridization and complementary sequences. Cell–material interactions can be programmed by switching the binding states of cells on a hydrogel surface using hybridized aptamers for cell-type-specific catch and triggering complementary sequences yielding dissociation of the aptamers from the hydrogel and simultaneous cell release. The programmable hydrogel is regenerable and can be applied to a new round of cell catch and release when needed. Adapted with permission.[77] Copyright 2012, American Chemical Society. c) DNA-based hydrogel for cell attraction, catch and killing of diseased cells (e.g., cancer cells) inspired by Drosera. The combination of a two-layered hydrogel is presented to afford a material with multiple functions that can be performed synergistically or sequentially. The hydrogel consists of two layers, the top layer is functionalized with oligonucleotide aptamers allowing for catching of target cells with high efficiency and specificity while the bottom layer hydrogel is functionalized with double-stranded DNA which can sequester intercalated drug for sustained drug release, killing the cells bound to the top layer in close proximity. Subsequent to cell release, the bifunctional hydrogel can be regenerated for continuous cell catching and killing. Adapted under the terms of the Creative Commons Attribution 4.0 International License.[76] Copyright 2015, The Authors.

together with the option for versatile biofunctionalization of DNA hydrogels makes them attractive as cellular scaffolds, e.g., for tissue engineering.

Despite the tremendous progress in the development of DNA-based hydrogels, several challenges need to be addressed before a wide-spread usage will become feasible. First, the cost for the production of DNA needs to be further reduced to allow for cost-effective upscaling. We also believe that a biotechnological approach might become suitable for producing DNA building blocks and even DNA nanostructures above a certain size,[4] but purification remains the critical issue. In contrast, synthetic methods are more suited for preparing short DNA fragments and especially when nonnatural functions need

to be incorporated. Most likely, a combination of improved biotechnological production and automated DNA synthesis will provide the pathways to sufficient amounts of material as seen for peptide solid-phase synthesis and protein expression. Second, by developing new chemical handles as well as by incorporating un-natural nucleotides, the toolbox of DNA functions could be expanded, which may lead to the discovery of completely new properties of DNA materials. For biomedical applications, the stability of the DNA constructs as well as their blood circulation and potential interactions with blood proteins need to be studied in greater details.

Overall, the unique programmability of DNA paves the way to smart therapeutic systems and we could imagine DNA

hydrogels as tissue-like components that locally detect changes in their environment and release distinct quantities of different therapeutics on demand. In this way, exciting future opportunities for personal medicine could be envisioned.

Conflict of Interest

The authors declare no conflict of interest.

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1906253 (25 of 25)