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Supramolecular Hydrogels Based on DNA Self-Assembly

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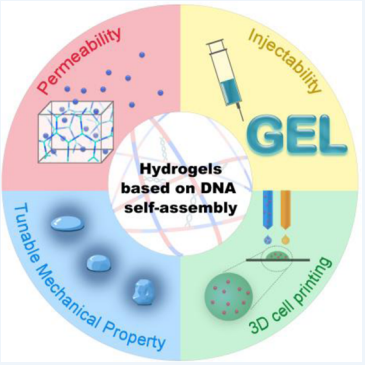
CONSPECTUS: Extracellular matrix (ECM) provides essential supports three dimensionally to the cells in living organs, including mechanical support and signal, nutrition, oxygen, and waste transportation. Thus, using hydrogels to mimic its function has attracted much attention in recent years, especially in tissue engineering, cell biology, and drug screening. However, a hydrogel system that can merit all parameters of the natural ECM is still a challenge.

In the past decade, deoxyribonucleic acid (DNA) has arisen as an outstanding building material for the hydrogels, as it has unique properties compared to most synthetic or natural polymers, such as sequence designability, precise recognition, structural rigidity, and minimal toxicity. By simple attachment to polymers as a side chain, DNA has been widely used as cross-links in hydrogel preparation. The formed secondary structures could confer on the hydrogel designable responsiveness, such as response to temperature, pH, metal ions, proteins, DNA, RNA, and small signal molecules like ATP. Moreover, single or multiple DNA restriction enzyme sites could be incorporated into the hydrogels by sequence design and greatly expand the latitude of their responses. Compared with most supramolecular hydrogels, these DNA cross-linked hydrogels could be relatively strong and easily adjustable via sequence variation, but it is noteworthy that these hydrogels still have excellent thixotropic properties and could be easily injected through a needle. In addition, the quick formation of duplex has also enabled the multilayer three-dimensional injection printing of living cells with the hydrogel as matrix. When the matrix is built purely by DNA assembly structures, the hydrogel inherits all the previously described characteristics; however, the long persistence length of DNA structures excluded the small size meshes of the network and made the hydrogel permeable to nutrition for cell proliferation. This unique property greatly expands the cell viability in the three-dimensional matrix to several weeks and also provides an easy way to prepare interpenetrating double network materials.



In this Account, we outline the stream of hydrogels based on DNA self-assembly and discuss the mechanism that brings outstanding properties to the materials. Unlike most reported hydrogel systems, the all-in-one character of the DNA hydrogel avoids the "cask effect" in the properties. We believe the hydrogel will greatly benefit cell behavior studies especially in the following aspects: (1) stem cell differentiation can be studied with solely tunable mechanical strength of the matrix; (2) the dynamic nature of the network can allow cell migration through the hydrogel, which will help to build a more realistic model to observe the migration of cancer cells *in vivo*; (3) combination with rapidly developing three-dimension printing technology, the hydrogel will boost the construction of three-dimensional tissues and artificial organs.

1. INTRODUCTION

The extracellular matrix (ECM) is a dynamic three-dimensional (3D) complex structure where cells reside, remodel, and interact over a range of length scales to maintain tissue homeostasis, growth, and repair; it is mainly composed of protein fibers, sugar, and high content of water.[1] In the last decades, hydrogels, which have a similar network topology and water content to the ECM, have emerged as the most popular scaffolds to reconstitute artificial 3D environments that impart biochemical and biophysical cues to regulate cell fate and functions.[2] Due to biocompatibility requirements, only a few synthetic polymers, like PEG, PLGA, etc., and natural polymers including proteins, DNA, and polysaccharide could be used as the backbone.[3,4]

The hydrogels could be divided into two types, according to their cross-linking methods: one type is "chemical hydrogels", which is the most commonly studied system.[5] Their polymer chains are cross-linked via covalent bonds, which bring them high mechanical strength, environmental stability, and shape-memory properties. However, the strong cross-linking also limits cell proliferation and migration and prevents their application in 3D cell culture. On the other hand, "physical hydrogels" rely on noncovalent supramolecular interactions such as hydrogen bonding, metal−ligand coordination, host−guest complexation, and electrostatic interactions to cross-link.[6−8] The dynamic nature of these interactions confers on hydrogels shear-thinning and self-healing properties and provides the possibility of cell migration. However, the

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application of hydrogels in 3D cell culture is still a great challenge because of poor nutrient permeability.

As illustrated in Figure 1A, the hydrogel network can be described as a group of cross-linking points with polymer

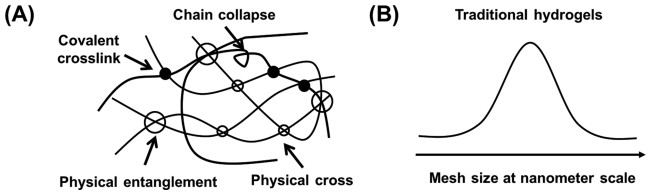

Figure 1. Traditional polymer network structure: (A) traditional polymer network chain conformation; (B) mesh size distribution of the traditional hydrogels.

chains connecting to each other. According to the preparation mechanism, the length of these connecting polymer chains should be in a random normal distribution. In the meantime, the persistence length of most polymer chains is less than nanometer scale, and chain curling, folding, wrapping, and collapsing add another variation on the distance distribution between cross-linking points. Subsequently, the mesh size of the hydrogel determined by these distances must have a random normal distribution from sub-nanometer to even micrometer scale (Figure 1B). It has been widely accepted that this distribution is one important factor to enable controllable release, where the small mesh size contributes the most. However, the same mechanism also prevents the nutrient molecules from going through the hydrogel network efficiently in 3D cell culture, especially the proteins of larger size. It is easy to elucidate that using a sufficiently long, rigid, water-soluble polymer as backbone could avoid the formation of small-size meshes in the network. However, design and synthesis of such polymers have long been an extreme challenge in polymer chemistry.

In the past 30 years, the DNA molecule has received considerable attention as a promising material because of its precise base-paring recognition, designable sequence, and predictable secondary structure. It has been widely used in preparing given-sized, precisely addressable, two- and three-dimensional nanostructures,[9] as well as nanomachines[10] and functional devices,[11,12] but not bulk materials. In 2006, Luo and his co-workers fabricated a chemically cross-linked DNA network by enzymatic ligation of branched DNA scaffolds, which has a relatively fixed cross-linking-point distribution.[13] This hydrogel exhibited amazing shape memory properties upon the reformation of duplexes in rehydration processes.[14] Related to the same reason, it might not provide the structural dynamics that is essential for 3D cell culture. In 2009, Liu's group used only short duplex or i-motif structures, which are rigid as their lengths are all shorter than their persistence length, to fabricate a new type of purely supramolecular hydrogel based on DNA self-assembly.[15] This novel hydrogel is dynamic, mechanically strong, and biocompatible and, especially, has excellent molecular permeability.[16,17] It has been successfully applied in 3D cell printing and was thought to be a most promising material for future 3D cell culture and tissue engineering.[18,19]

In this Account, we focus on the hydrogels based on DNA self-assembly and summarize their preparation strategies in Figure 2. According to their composition, these hydrogels are categorized in two groups: (1) hybrid DNA supramolecular

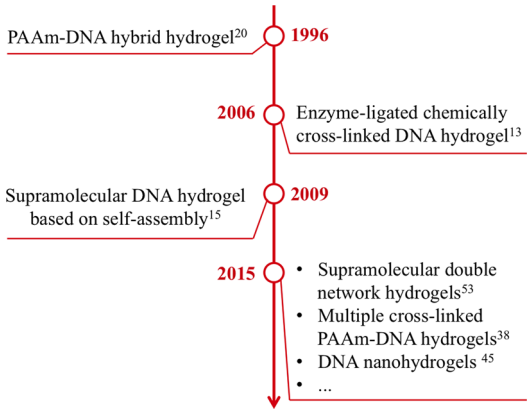

Figure 2. Development of supramolecular DNA hydrogel system.

hydrogels using synthetic or natural polymers as backbone and DNA assemblies as cross-linking points; (2) pure DNA supramolecular hydrogels that are built by branched and linear DNA assembled scaffolds upon rational design. In each part, we will summarize the origin design, and strategy optimization, preparation, and characterization, mechanism, and applications (Table 1) of the hydrogels and try to find out future direction in this field.

Table 1. Summary of Supramolecular DNA Hydrogel Systems

types	hybrid DNA hydrogels	pure DNA hydrogels
	Responsiveness	
pH	Liu and Deng et al.;[27] Willner et al.[38]	Liu et al.[15,48,52]
temperature	Nagahara and Matsuda;[20] Langrana et al.[21]	Liu et al.[16]
enzyme	Liu and Weil et al.[24]	Liu et al.;[16] Lei et al.[54]
ion	Liu et al.[23]	
mechanics	Liu et al.[34]	Liu et al.[52]
aptamer	Mi et al.;[28] Tan et al.;[29,30] Liu et al.;[31] Yang et al.;[32]	Tan et al.;[45] Lei et al.[54]
	Applications	
multiple functionalization	Liu et al.[35]	
3D printing	Liu and Shu et al.[18]	
cell culture	Liu and Shu et al;.[18] Liu and Weil et al.[24]	Liu et al.;[17] Tan et al.[45]
diagnostics	Tan et al.;[30] Liu et al.;[31] Yang et al.[32]	Lei et al.[54]
drug delivery	Liu and Weil et al.;[24] Wang et al.;[25] Mi et al.[28]	Tan et al.;[45] Ke et al.;[47] Nie and Huang et al.[55]
shape memory	Willner et al.[38−40]	Luo et al.[13,14]
double network		Liu et al.[53]

2. HYBRID HYDROGELS BASED ON DNA SELF-ASSEMBLY

In the hybrid DNA hydrogels, short DNA sequences are grafted onto the backbones physically or chemically. The hydrogels are formed via the formation of DNA assembled structures. Reported backbone materials include polyacrylamide,[20,21] polypeptide,[22,23] protein,[24] poly(phenylenevinylene),[25] graphene oxide,[26] carbon nanotube,[27] etc. In these hydrogels, only a small quantity of DNA is required, and self-assembled DNA acts as both the cross-linkers and switchable elements. Because the stability of DNA secondary structures is better than

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small-molecule supramolecular interactions, the resulting hydrogels are normally much stronger. In the meantime, this type of hydrogel can possess different responsiveness and be easily functionalized because of variable sequence design of the DNA.

2.1. Strategies

In 1996, Nagahara and Matsuda[20] reported the first polyacrylamide−DNA hydrogel. Through an active ester-amine click reaction, 5′-terminal-amino-modified DNA chains were coupled with a poly(*N*,*N*-dimethylacrylamide-*co*-*N*-acryloyloxysuccinimide). The DNA side chains of prepared water-soluble copolymers then hybridized with complementary DNA strands to form a network. The DNA-induced cross-linking was reversible when temperature changed (Figure 3A).[20] In 2004,

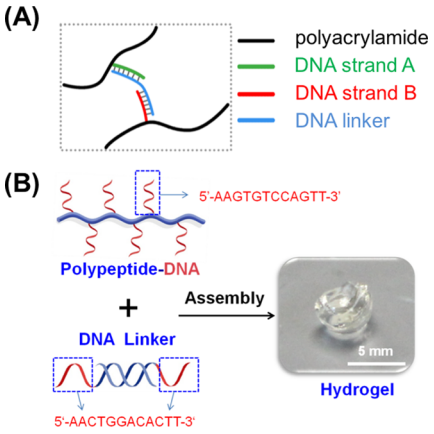

Figure 3. DNA-functionalized polymer hydrogels as stimuli-responsive materials: (A) formation of a reversible, DNA cross-linked polyacrylamide hydrogel and the diffusion-driven dissociation of the polymer network by removal of a DNA strand; (B) formation of polypeptide−DNA hydrogel based on DNA self-assembly.

Langrana's group[21] employed 5′-acrydite-modified-DNA copolymerized with acrylamide in buffer solution. By adding an overhang onto the linker DNA sequences, the hydrogel could also be switched to solution by adding competing full-length complementary sequences of linker in addition to thermal responsiveness.[21] Afterward, a series of hydrogels achieved using DNA aptamers as cross-linker and their applications in diagnostics have been explored.[28−32]

In recent years, polypeptides and proteins as promising biomaterials have received great interest because of their biodegradability and biocompatibility. In 2015, Liu, Li, and their co-workers[18] reported a method to prepare DNA grafted polypeptides via an azide−alkyne click chemistry and prepared a DNA hybrid polypeptide hydrogel by hybridization with linker DNAs (Figure 3B). The mechanical strength of this supramolecular hydrogel was significantly enhanced.[18] In 2014, Liu, Weil, et al.[24] used a PEGylated, denatured human serum albumin (HSA) as backbone to prepare a protein−DNA hybrid hydrogel. Cell viability experiments demonstrated that this hydrogel has excellent cell compatibility. They also showed that DNase I and trypsin could easily degrade the gel within 30 min.[24]

Besides chemical modification, DNA single strands can also interact with polymers via physical interactions (electrostatic interaction, $\pi-\pi$ interaction, etc.). In 2009, Wang et al.[25] mixed positively charged, water-soluble poly(phenylenevinylene) with double stranded DNAs to yield a physical hydrogel. The conjugated polymer made the hydrogels fluorescent, which could be used to monitor drug release.[25] Through rational sequence design, Liu, Deng, et al. employed a GT-rich DNA single stranded domain to effectively attach a cytosine-rich DNA sequence onto carbon nanotubes via $\pi-\pi$ interaction.[27] Upon pH trigger, a transparent DNA/carbon nanotube supramolecular hydrogel could be prepared.[27,33] In 2010, Shi's group annealed water-soluble graphene oxide with double stranded DNA. During the thermal treatment, the DNA partially disassociated and the single-stranded part could attach to the graphene oxide via $\pi-\pi$ interactions and formed a hydrogel accordingly.[26] These strategies not only provide new methodology to prepare DNA supramolecular hydrogels but also confer fascinating responsiveness on the hydrogels.

2.2. Mechanical Properties

The formation of DNA secondary structures is based on multivalent interactions including hydrogen bonding, $\pi-\pi$ stacking, and hydrophilic/hydrophobic interactions. Due to this mechanism, the hydrogels based on DNA self-assembly are normally much stronger than those based on simple supramolecular interactions; in the meantime, they also maintain all the unique mechanical properties of supramolecular hydrogels such as self-healing and thixotropic properties. Furthermore, the designability of DNA sequences enables the preparation of hydrogels with identical cross-linking point density but distinctive rheological properties. Recently, Liu and co-workers demonstrated that, in a DNA−polypeptide hydrogel system, the storage moduli (G') of the material were positively related to the stability of DNA linkers (Figure 4A). By extending or shortening the length of DNA linker or inserting mismatch sites, the G' values of these hydrogels could be varied from several thousands to several tens of pascals (Figure 4B).[34] This property provides a practical platform to study the cellular response to mechanical signals within a three-dimensional environment.

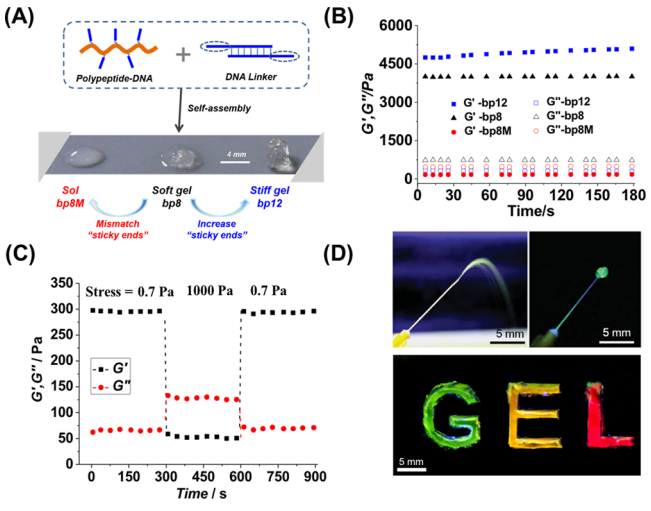

Figure 4. Modulation of mechanical properties of polypeptide−DNA hydrogel: (A) tuning mechanical properties by tailoring the length of sticky ends or introducing mismatch; (B) rheological test of polypeptide−DNA hydrogels with different mechanical strength; (C) rheological properties of 4 wt % polypeptide−DNA hydrogels with an alternating stress of 0.7 and 1000 Pa with a fixed frequency (1 Hz) at 25 °C for 5 min; (D) injection behavior of hydrogels via syringe and direct writing of modified hydrogels into 3D pattern of "GEL".

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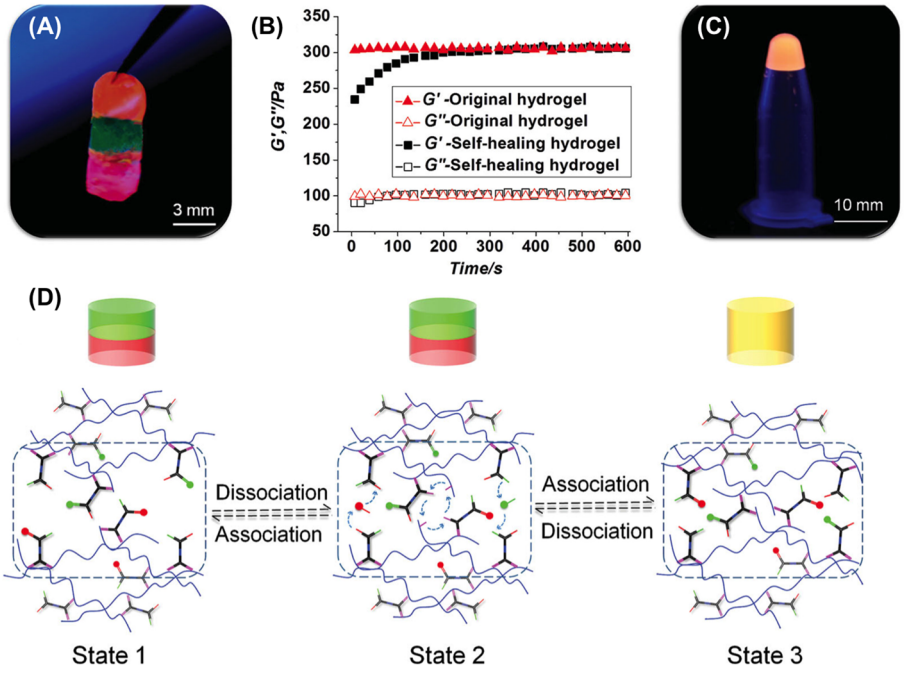

Figure 5. Self-healing properties of freshly prepared polypeptide–DNA hydrogels: (A) freshly prepared hydrogels (4 wt %) can stick to each other and can be manipulated by tweezers; (B) mechanical property recovery of healed polypeptide–DNA hydrogel; (C) three adhered hydrogels modified with different dyes healed into each other and exhibited an intermediate orange color after being left at 4 °C overnight; (D) DNA chain exchange mechanism of self-healing.

Even though DNA secondary structures are very stable at physiological conditions, they are still reversible upon thermal, chemical, and mechanical triggers. Thus, the hydrogels based on DNA self-assembly are reversible and have excellent thixotropic property. As shown in Figure 4C, under relatively high oscillatory stress (1000 Pa), the 4 wt % polypeptide–DNA hydrogel turned into a quasi-liquid state at room temperature; when the stress changed to 0.7 Pa, the system could return to the original gel state within seconds. This transition allows the hydrogel to be easily injected through a needle. Moreover, the rapid sol–gel phase transition provides outstanding shaping ability after injection, which allows the gel to be written into arbitrary three-dimensional shapes (Figure 4D).[35]

Also based on the dynamic properties of DNA secondary structures, the hydrogels possess extraordinary self-healing or self-repairing properties. In 2015, Liu's group[35] found that two pieces of DNA–polypeptide hydrogels could easily stick to each other (Figure 5A). As shown in Figure 5B, the mechanical strength of the merged material could reach 80% of their original value in seconds and was totally recovered in 5 min. With different fluorophore labeling, they also demonstrated that the self-healing was based on chain-exchange reaction of DNA double helix (Figure 5D). Upon DNA chain diffusion, the interfaces between different parts could be eliminated; after 24 h, the three differently colored hydrogels merged thoroughly and adapted themselves into the container's shape (Figure 5C).[35]

2.3. Multiple Functionalization

Generally, hydrogels need to be modified by functional molecules chemically or physically to fulfill the requirement of applications. However, these two methods both have some drawbacks such as time- and labor-consuming, costly synthesis and purification processes, unwanted aggregation inducing uneven distribution of functional molecules, etc. It is noteworthy that, by rational design, branched DNA scaffolds could be easily prepared and have been widely used to fabricate two-dimensional lattices[36] and three-dimensional dendrimers.[37] In 2015, Liu and co-workers employed X-shaped DNA scaffolds to replace the linear DNA linkers in DNA–polypeptide hydrogels.[35] As shown in Figure 6, there are four sticky ends on the

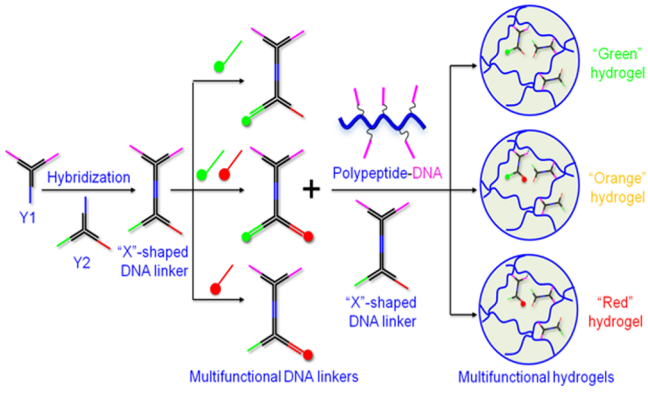

Figure 6. Polypeptide–DNA hybrid hydrogels with rationally designed multimodification sites. The "X"-shaped DNA linker contains four "sticky ends"; the two pink sticky ends are used for the formation of hydrogel network. The other two are different sequences (green and red) that are free to be hybridized with their complementary sequences with specific dye modification.

X-shaped DNA scaffold: two of them are identical and are complementary to the DNA strands grafted on the polypeptides; the other two are different and work as the functionalized docks. According to this design, different functional groups conjugated with corresponding complementary DNA strands can be attached onto the hydrogel backbone via precise DNA recognition, and their distribution is uniform

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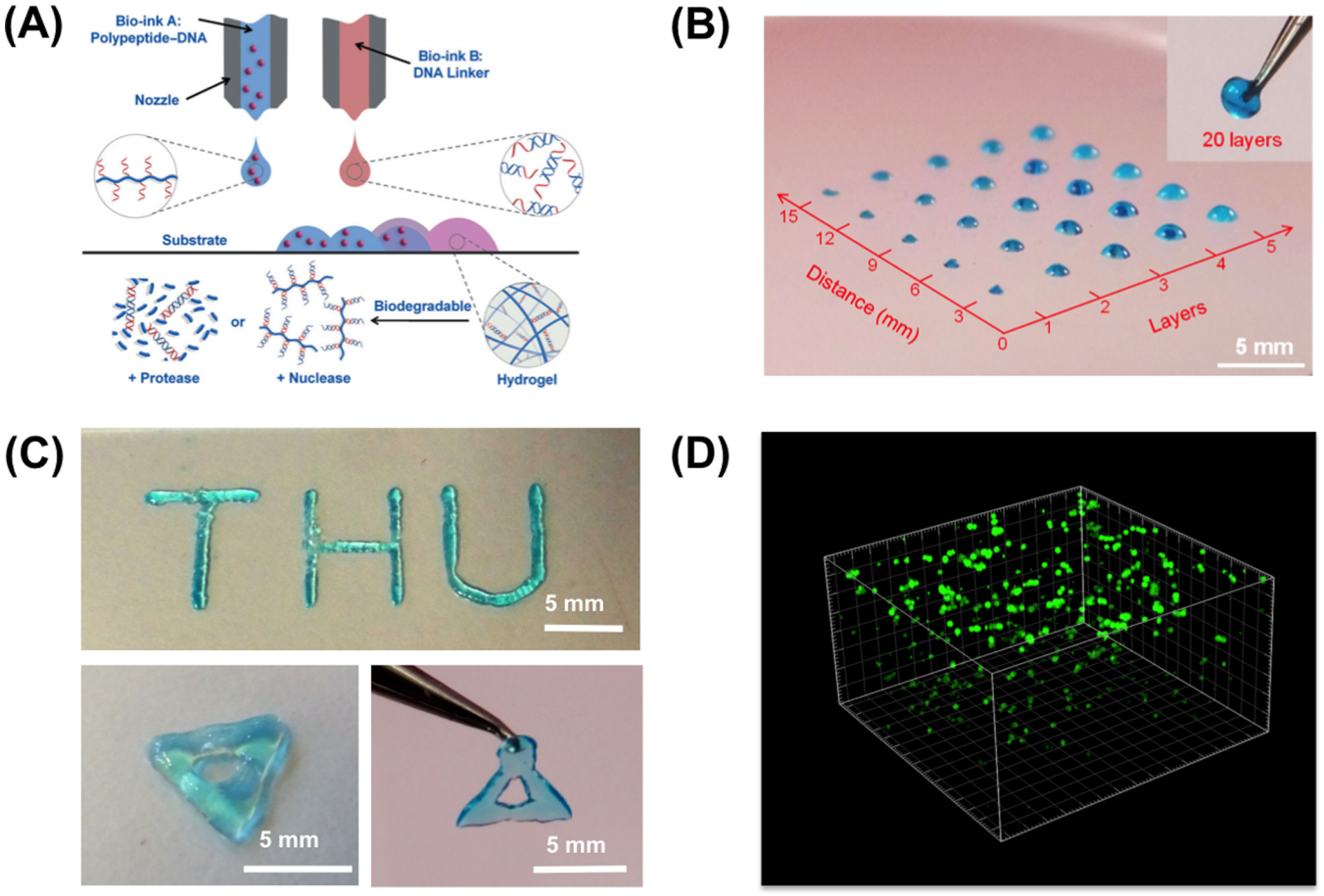

Figure 7. Rapid formation of a supramolecular polypeptide–DNA hydrogel for *in situ* three-dimensional cell printing: (A) 3D bioprinting of the polypeptide–DNA hydrogel to fabricate arbitrarily designed 3D structures; (B) array of printed droplets with an increasing number of layers (up to 5 layers), from left to right, layer number increased, and the multiple layer dots are formed via repeating printing layer-by-layer. Inset: Hydrogel structure with 20 layers; (C) The letters "THU" printed with five layers and a triangle with ten layers, which is strong enough to be picked up with tweezers; (D) 3D stacks of AtT-20 cells printed in a hydrogel with FDA staining in green (gridlines = 50 mm).

because of the charge repulsion and structural rigidity of DNA structures. It should be mentioned that this strategy could be easily expanded to multiple functional docks by using multibranched DNA scaffolds, and the ratio between different functional molecules could also be predetermined.

2.4. Dual Cross-Linking and Shape Memory

In the above-mentioned examples, there is only one type of DNA strand attached to the polymer main chain. However, current modification chemistry can permit more than that. Recently, Willner et al. took this advantage and reported a multitriggered DNA-based hydrogel. The polyacrylamide chain was modified with two types of DNA single strands, which can form i-motif and DNA duplex under suitable conditions. These two kinds of formed DNA secondary structures cross-linked the polyacrylamide chains cooperatively at pH 5. When pH was changed to 8, the i-motif structures disassociated and the cross-linking density of the hydrogel decreased significantly. Under optimized conditions, the mechanical strength of the system will decrease accordingly and lead to the transition from stiff gel to a quasi-solution. In the meantime, the existence of duplex DNA cross-linkers can roughly maintain the integrity of network and keep the disassociated i-motif DNA strands spatially close; when pH was adjusted to 5 again, these nearby strands can form bimolecular i-motif structures again and make the network return to its original organization in general.[38] Under certain time and temperature range, the bulk hydrogel exhibited a pH-responsive shape-memory behavior. Selectively switching one DNA assembled structure but keeping the others untouched explored a new way to facilitate smart hydrogels, which was demonstrated a general principle in the following studies.[39,40]

2.5. Three-Dimensional Cell Printing

Recently, three-dimensional bioprinting has emerged as a powerful fabrication method to create tissue-like structures.[41] To make the cells survive through the printing process, the ink materials must meet many strict requirements, such as very low viscosity during printing, rapid gelation under mild conditions, and sufficient mechanical strength for self-support. Each requirement is feasible, but fulfilling all of them at the same time with a single hydrogel has been a great challenge for many years. In 2015, Liu, Shu, et al. demonstrated that the supramolecular polypeptide–DNA hydrogels worked perfectly with the dual-nozzle printer and enabled *in situ* multilayer three-dimensional living cell bioprinting.[18] As shown in Figure 7A, polypeptide–DNA solution mixed with living cells acted as one ink, and the complementary DNA linker solution is another one. Because their concentrations were both very low, the viscosities of each ink could be low enough to effectively decrease the nozzles' working pressure, as well as the shearing forces applied on the cells. It has been demonstrated that the

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formation of DNA secondary structures could be finished within 1 s if diffusion is fast enough.[42] Printing at nanoliter scale guaranteed the thorough and quick mixing of the two inks and led to the formation of free-standing hydrogels. By repeating the printing program, three-dimensional tissue-like structures with desired scales and dimensions could be constructed (Figure 7B,C). The cells were embedded into the network during the process and distributed evenly (Figure 7D). It is worth noting that the printed structures are optically transparent and geometrically uniform without obvious boundaries between the printed layers because of the self-healing property of the supramolecular hydrogel based on DNA self-assembly. It was claimed that, owing to cross-linking by the rigid DNA duplex, no obvious shrinking or swelling has been observed during the printing process, which avoids the possibility of shape deformation after printing. They characterized the viability and function of AtT-20 cells after printing and found about 98.8% cells were viable, had normal 3D morphology, and exhibited various cellular functions, including proton pump activity, metabolic turnover, and membrane trafficking.[18] This research illustrated that the supramolecular hydrogels based on DNA self-assembly is a new promising printable biomaterial for the fabrication of complex three-dimensional tissue-like constructs in tissue engineering.[19]

3. PURE DNA HYDROGELS BASED ON DNA SELF-ASSEMBLY

Pure DNA hydrogels are formed by branched and linear DNA assembled scaffolds (Figure 8A). According to the same mechanism, this type of hydrogel inherits all of the unique properties of previously described hybrid DNA supramolecular hydrogels. In most cases, the distance between cross-linking points is shorter than the persistence length of B-helix of double stranded DNA,[43,44] which makes the network rigid and the smallest meshes are excluded (Figure 8B). Thus, the hydrogels are permeable to small molecules and proteins around 22 kDa.

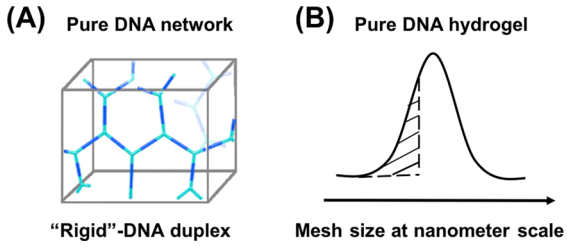

Figure 8. Pure DNA network structure: (A) pure DNA network chain conformation; (B) mesh size distribution of the pure DNA hydrogels.

3.1. Strategies

In 2009, Liu, Zhou, and co-workers reported the first pure DNA supramolecular hydrogel formed by only three 37-mer DNA strands using a one-pot stepwise self-assembly protocol.[15] As shown in Figure 9A, the three sequences were partially complementary to each other and could assemble into a Y-shaped DNA nanostructure at pH 8. This Y-scaffold has a rigid double stranded central domain and three half i-motif sequences dangling out at all ends as interlocking domains. Then the pH value is lowered to 5 to let the intermolecular i-motif structures form between scaffolds to form the hydrogel. Because the core structure of Y-scaffolds is rigid, similar i-motif structures cannot form inside the scaffold. This is the first hydrogel example that was purely based on DNA self-assembly. According to literature,[42] the formation of i-motif structures was reversible and rapid; therefore such a hydrogel could be switched between gel and solution states within 1 min by adjusting the environmental pH.

It is interesting to notice that authors employed 13 nm gold nanoparticles to illustrate the controllable release ability of hydrogel, because small molecules, like fluorescein, could not be trapped. In the following studies, they reasoned that there are only duplex and i-motif structures between cross-link points of the network, and both of them are shorter than their own persistence length. The all-"rigid" network eliminates the small meshes and lets the small molecules go through freely. Other than preventing the self-collapse, the rigidity of the network also inhibits the elongation of the distances between cross-links, which makes the hydrogel nonswelling.

The unique permeability might have important application in three-dimensional cell culture, but the lower pH environment limited its application. In 2011, Liu et al. reported a pure DNA hydrogel based on DNA self-assembly that was formed and stabilized under physiological conditions.[16] This new system was composed by two types of building blocks, the Y-scaffold and a linear linker (Figure 9B); simply mixing these two components at a proper ratio would induce the formation of hydrogel by the hybridization of sticky ends. The length of linear linker could be varied in the several tens of base pairs range; thus one or more restriction sites could be accommodated, bringing specific enzymatic responsiveness to the hydrogel.

In 2015, Tan and co-workers[45] reported the preparation of a nanohydrogels based on DNA self-assembly. They introduced a

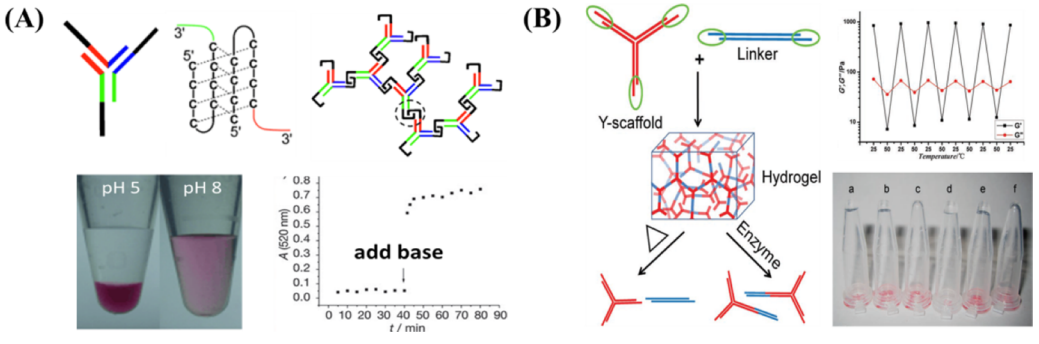

Figure 9. Self-assembled DNA hydrogels: (A) pH triggered DNA hydrogels for controlled gold nanoparticle release; (B) DNA hydrogels with designable thermal and enzymatic responsiveness.

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new Y-shaped unit with only one sticky end, which limited the network expansion. By changing the ratio of the two Y-shaped units, the size of the nanohydrogels could be controlled in the nanometer scale. In the study, they also incorporated different functional elements, such as aptamers, disulfide linkages, and therapeutic genes into building units, which conferred stimuli-responsiveness to the nanohydrogel.[45]

In 2014, Paululat, Noll, et al. reported a strategy that could use two partially complementary sequences to prepare a hydrogel.[46] In 2016, Ke's group[47] pushed this bar to the top: through rational design, they used a single DNA strand to prepare a supramolecular hydrogel purely based on DNA self-assembly. The strand contained three or more domains that could form branched assembly structures through a careful annealing process.[47] Based on similar strategy, Liu et al. reported a pH triggered, fast-forming DNA hydrogel formed by one single stranded DNA in a two-step procedure.[48] The single-stranded strategy avoids the quantitative match of every sequence and thus can reduce labor cost and improve the homogeneity between different batches.

3.2. Mechanical Properties

Similar to the strategy described in the hybrid system, varying length and composition of sticky ends could vary the mechanical propertiees of pure DNA hydrogels. However, the structural response to stimulus of DNA has been used to facilitate molecular machines,[10,49] which can give a 5 pN force output and bend a microcantilever.[50,51] By sequence design, these molecular machines can be easily incorporated into the pure DNA hydrogel network. In 2016, Liu's group[52] reported a DNA hydrogel with pH driven DNA molecular motors as part of the linear linker (Figure 10). When the pH values were

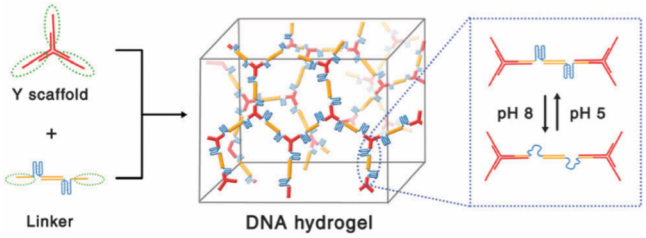

Figure 10. Tunable mechanical properties of pure DNA hydrogel with pH responsiveness.

switched between 5 and 8, the storage modulus of hydrogel was swiftly changed between 1000 to 250 Pa accordingly.[52] They demonstrated that the transition was mainly based on the movement of DNA motors, which is fast and reliable.[42] As the responsiveness is triggered by the conformational change of specific DNA analogs, we can propose that this strategy can enable the preparation of smart hydrogels responding to different stimuli, such as potassium ions, ATP, etc., by adopting corresponding DNA sequences.

3.3. Double Network Hydrogels

The base-pair recognition of DNA is distinct from other reported supramolecular interactions, and they have very little possibility to influence each other. In 2015, Liu, Scherman, et al. took advantage of this orthogonality to prepare a pure supramolecular double network hydrogel.[53] As illustrated in Figure 11, cucurbit[8]uril (CB[8]) and Y-scaffold were premixed in PBS buffer and then formed the hydrogel with the mixture of the other two components. Due to the quick formation of DNA networks, the aggregation and self-collapse of polymers were greatly hindered, and the formed double network hydrogel was demonstrated to be uniform by fluorescent microscopy. The physical interpenetrating and binding within each other contributed to the increased mechanical properties and thermal stability. However, the double network hydrogel possesses shear-thinning and thixotropic properties because it is entirely based on dynamic and reversible supramolecular interactions. Due to the natural properties of the biopolymer, the double network hydrogel exhibited dual-biodegradability to nuclease and cellulose, as well as to small molecules, enabling either selective partial or full degradation to meet different requirements for applications. It is believed to have great potential as injectable soft materials for *in vivo* applications.

3.4. Applications

To verify the biocompatibility of DNA hydrogels, Liu, Wang, et al.[17] positioned single cells in microwells and then sealed them with DNA hydrogel as a cover. The thickness of the cover is about 200−300 μm (Figure 12A).[17] They demonstrated that the cover was stable and the trapped cells could not be washed away by simple rinsing. After 24 h culturing, 98% ± 1% of the cells remained alive. Additional experiments verified that each scaffold and the hydrogel all have excellent cell compatibility. To confirm the permeability of hydrogel cover, they used fluorescent molecules as probes and monitored their diffusion course through the hydrogel cover with a confocal laser microscope. Results showed that the small molecule sodium fluorescein could go through the network very quickly and reach equilibrium in minutes. It should be emphasized that the 22 kDa molecule weight green fluorescent protein (GFP) could also go through the hydrogel cover in about 10 min (Figure 12B). This property could let commonly used nutrients in cell culture to reach the cells embedded in the DNA hydrogels and

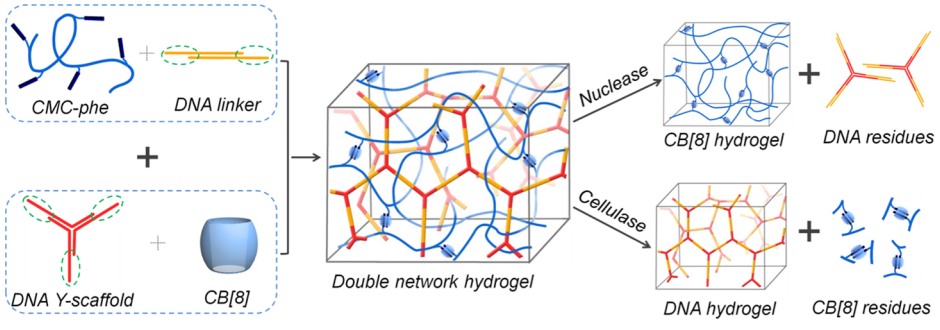

Figure 11. Responsive double network hydrogels of interpenetrating DNA and CB[8] host−guest supramolecular systems.

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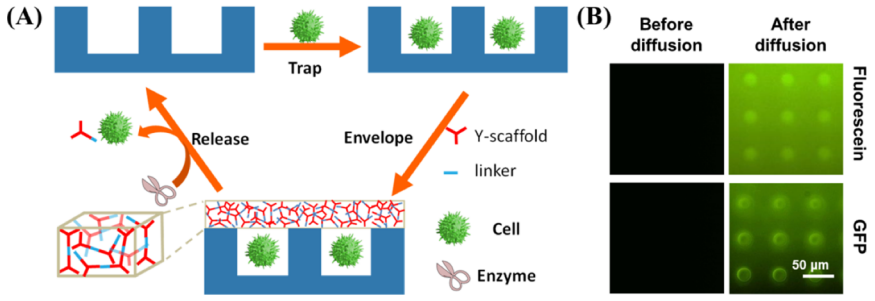

Figure 12. (A) A triggered DNA hydrogel cover to envelop and release single cells. (B) Fluorescence intensity in microwells covered with DNA hydrogel before and after immersed in sodium fluorescein or GFP protein solution. These data confirm that the DNA hydrogel is permeable and keeps cells viable.

makes three-dimensional cell culture possible. After observation, the DNA hydrogel cover could be specifically digested by a restriction enzyme, and the enveloped cells could be released controllably.

Besides cell culture, the application of assembled supramolecular DNA hydrogels in diagnostics and protein encapsulation have also been reported.[54,55] We believe that more applications of DNA hydrogels will be explored, especially based on their unique mechanical and permeable properties.

4. CONCLUSION AND PROSPECTS

In summary, the unique rigidity of DNA double helixes and their designable sequences push hydrogel research to a new level: these new supramolecular hydrogels have natural biocompatibility and biodegradability; through rational design, the hydrogel could be easily functionalized and endowed with multiple responsiveness; because of their fast *in situ* formation under physiological conditions and good mechanical properties, supramolecular hydrogels based on DNA self-assembly have been demonstrated to be exceptionally compatible with three-dimensional living cell bioprinting techniques; the unbending DNA structures eliminate small meshes in the network and ensure extraordinary molecule permeability, which guarantees the high cell viability in three-dimensional cell culture. Most of these properties have been reported before in different systems individually but have never been observed from one material. The all-in-one character of the DNA hydrogel avoids the "cask effect", and we expected it will benefit research on cell behavior and tissue engineering in three dimensions, especially on the following detailed aspects: first, stem cell differentiation can be studied with solely tunable mechanical strength of the matrix; second, the dynamic nature of the network can allow cell migration through the hydrogel, which will help to build more realistic models of migration of cancer cells *in vivo*; third, combined with rapidly developing three-dimensional printing technology, the hydrogel will boost the construction of three-dimensional tissues and artificial organs. We can also imagine that this material will have wide clinical application in tissue repair, where its injectability will minimize the invasion required for therapies and greatly reduce the patients' suffering.

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Notes

The authors declare no competing financial interest.

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