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Programmable Macroscopic Self-Assembly of DNA-Decorated Hydrogels

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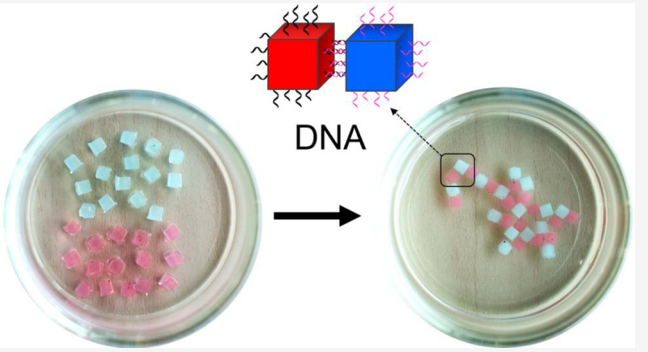
ABSTRACT: The precise and predictable formation of double-helical structures from complementary DNA sequences has made DNA an extremely versatile tool for programming self-assembled structures from the nanometer to micrometer scale. While a number of supramolecular interactions have been shown to drive self-assembly of macroscopic building blocks of the millimeter scale, DNA-driven self-assembly of macroscopic objects has not been well-established. In this work, we developed a postpolymerization coupling strategy to conjugate short DNA sequences to polyacrylamide-based hydrogel blocks. We observed sequence-specific self-assembly of DNA-decorated hydrogels with 1−2 mm edges in aqueous solution. Furthermore, selective disassembly of hydrogels upon addition of a DNA strand was demonstrated by exploiting a strand displacement reaction. These results lay the foundation for adaptation of various DNA functions to macroscopic self-assembly, for example, molecular recognition, molecular computation, and chemical catalysis.



INTRODUCTION

Arguably, DNAs possess ideal chemical properties for molecular self-assembly.[1] Oligo DNAs with complementary sequences form a duplex with predictable stability and sequence-independent structure. A large number of orthogonal DNA pairs that specifically recognize each other can be designed easily, enabling remarkably complex self-assembly of thousands of DNA strands at the nanometer to micrometer scale (i.e., DNA origami).[2,3] DNAs can be immobilized on materials of the nanometer to micrometer scale such as hydrogels,[4] polymer beads,[5−7] gold nanoparticles,[8,9] vesicles,[10,11] and cells[12−15] to program their assembly. DNAs can also be designed to form polymeric networks or can be incorporated into conventional linear polymers as cross-linkers to yield bulk hydrogels.[16,17] Another important benefit of such DNA-directed self-assembly is the ability to dynamically control specific DNA−DNA interactions using other DNA sequences, for example, through strand displacement reactions.[18]

Conspicuously missing from the diverse examples of DNA-directed self-assembly is that of macroscopic (millimeter or larger) objects.[19] Macroscopic self-assembly driven by supramolecular interactions was first demonstrated by Harada et al., who reported self-assembly of millimeter-sized hydrogels that display cyclodextrin hosts and their guest moieties.[20] Since then, the Harada group and others have reported macroscopic self-assembly driven by electrostatic interaction,[21−23] metal−ligand interaction,[24] and hydrogen bonding.[25,26] In addition to the fundamental implications of the mechanistic basis of how molecular interactions define the assembly of macroscopic objects, macroscopic self-assembly may find novel applications,[27,28] for example, in regenerative medicine, by enabling synthesis of complex tissue structures through programmed assembly of cell-laden hydrogels.[29−32] Such complex self-assembly at the macroscopic scale would require predictable and programmable interactions such as those demonstrated by DNA-directed self-assembly.

In this work, we report programmed self-assembly of millimeter-sized hydrogels decorated with oligo DNAs. We synthesized polyacrylamide-based gels of 1−2 mm in dimension that bear azide ($-N_3$) functional groups. Oligo DNAs modified with alkyne groups at the 5′-ends were then coupled to the gel surface via a Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAC) reaction. These DNA-modified gels were analyzed for their ability to self-assemble in a buffer solution mediated by DNA hybridization (Figure 1).

RESULTS

Preparation of DNA-Decorated Hydrogels. We prepared hydrogel cubes by copolymerization of acrylamide

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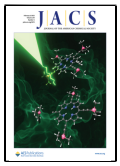

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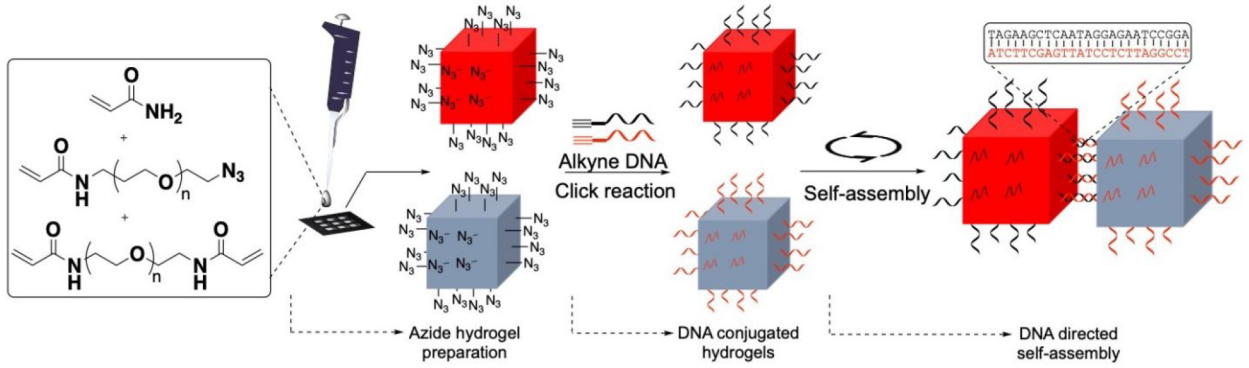

Figure 1. Preparation of DNA-decorated hydrogels and their self-assembly. Polyacrylamide hydrogels containing azide ($-N_3$) groups are produced by radical polymerization. The hydrogels are then reacted with 5′-alkyne-modified oligo DNAs to yield DNA-decorated gels. The hydrogels displaying complementary DNA sequences self-assemble via DNA hybridization.

(AAm, 5.5% w/v) and acrylamide−PEG2K−azide (AAm−PEG2K−N_3, 4% w/v) as monomers and PEG1K−bis-(acrylamide) (BAAm−PEG1K, 1% w/v) as a cross-linker. Standard ammonium peroxodisulfate (APS)-catalyzed polymerization at 25 °C in custom-made molds yielded transparent hydrogel cubes or cuboids containing azide groups (Figure 1).

Oligo DNAs modified with 5′-alkyne groups were conjugated to the azide hydrogels via a CuAAC reaction to produce DNA-decorated hydrogels. A 1.5 mm hydrogel cube was reacted with 0.25, 1.0, 1.5, and 2.0 nmol of 5′-alkyne-modified DNA ON-1. To confirm DNA conjugation, an equivalent amount of fluorescein amidite (FAM)-labeled DNA that is partially complementary to ON-1 (FAM-ON-1C, Figure 2a, and Table S1) was allowed to hybridize with the gel-immobilized ON-1 in 50 μL of phosphate-buffered saline (PBS, pH 7.5). Each hydrogel was washed three times with PBS to remove unhybridized FAM-ON-1C. The ON-1-decorated gel showed intense fluorescence upon excitation at 365 nm, while the gel modified with ON-1C (complementary to ON-1) and the unmodified gel showed no visible fluorescence (Figure 2b). The captured FAM-ON-1C was dissociated by heating the hydrogel to 80 °C, and the eluted FAM-ON-1C was quantified by fluorescence. The 1.5 mm cubic hydrogels were able to hybridize with 0.12 ± 0.02, 0.42 ± 0.01, 0.58 ± 0.02, and 0.88 ± 0.05 nmol of FAM-ON-1C when reacted with 0.25, 1.0, 1.5, and 2.0 nmol of 5′-alkyne-modified ON-1, respectively (Figure S1). A gel reacted with 2 nmol of DNA was sliced and imaged upon excitation at 365 nm to visualize the spatial distribution of FAM-ON-1C. Intense fluorescence was observed near the cube's surface, suggesting that the majority of the immobilized DNA was localized on the surface (Figure 2c). Analysis by scanning electron micrography (SEM) of the hydrogel surface before and after DNA conjugation revealed no significant differences (Figure S2).

Macroscopic Self-Assembly. We prepared 1 mm hydrogel cubes decorated with either ON-1 (25-mer) or its complementary sequence ON-1C following the above protocol (with 1.25 nmol of DNA used to immobilize per hydrogel). The ON-1 and ON-1C cubes were colored green and red, respectively, by adding chemically inert colored microbeads during polymerization. A total of 32 cubes were submersed in the assembly buffer (PBS, pH 7.5, supplemented with 500 mM NaCl, 5 mM $MgCl_2$, 10% w/v PEG 8000, and 0.01% Tween 20) and were shaken horizontally in a 30 mm dish. The hydrogel cubes self-assembled within 10 min. Remarkably, self-assembly was only observed between green and red cubes, with no interactions observed between the cubes of the same color (Figure 2d,e and Movie S1). A similar experiment using hydrogel cubes immobilized with 12-mer DNA showed only partial aggregation within 20 min (Figure S3) indicating that the observed assembly is dependent on the stability of the DNA duplex. Addition of hydrogel cubes (gray) decorated with ON-2 (25-mer) with no complementarity to ON-1 or ON-1C did not affect self-assembly, and the ON-2 gels remained unassembled, further demonstrating that the observed self-assembly is driven by sequence-specific DNA hybridization (Figure 2f,g and Movie S2). Addition of TURBO DNase (40 U/mL) to the self-assembled gels resulted in deaggregation (Figure S4).

To study the effect of DNA density on self-assembly, we prepared 1.5 mm cubes decorated with ON-1 (green) and ON-1C (red) using 0.25, 1.0, 1.5, and 2.0 nmol of DNA per hydrogel during the conjugation reaction. Self-assembly of the resulting hydrogels was monitored for 15 min (Figure S5). The hydrogels with the lowest amount of immobilized DNA (0.25 nmol/hydrogel) showed no self-assembly, while the hydrogels with the highest amount of immobilized DNA (2.0 nmol/hydrogel) assembled into a single aggregate. The hydrogels immobilized with 1.0 and 1.5 nmol of DNA showed partial assembly under the condition, indicating that the self-assembly depends on the density of the surface-conjugated DNA.

Next, we investigated the temperature dependence of self-assembly. Hydrogel cubes (1 mm) were decorated with ON-4 (green) and ON-4C (red) using 1.25 nmol of DNA per cube as described above. Eight ON-4 and eight ON-4C cubes were self-assembled for 15 min at 10, 25, 35, and 50 °C. The hydrogels rapidly assembled at 10 and 25 °C within 1.5 and 5 min, respectively (Figure S6a,b). At 35 °C, slow assembly was observed, with multiple aggregates of 2−5 cubes and several unassembled cubes (Figure S6c). No assembly was observed at 50 °C (Figure S6d). These results are consistent with the expected temperature dependence of DNA hybridization.

We then asked if multiple pairs of complementary DNAs can direct macroscopic self-assembly in parallel. In addition to ON-1/ON-1C, three pairs of 25-mer DNAs (ON-2/ON-2C, ON-3/ON-3C, and ON-4/ON-4C, Table S1) were designed and conjugated to 1 mm hydrogel cubes. Each pair was marked with differently colored microbeads to distinguish the gels. Starting with eight cubes per pair, the four pairs of hydrogels quickly self-sorted into distinct aggregates (Figure 3 and Movie S3) with one cube remaining unassociated probably due to the

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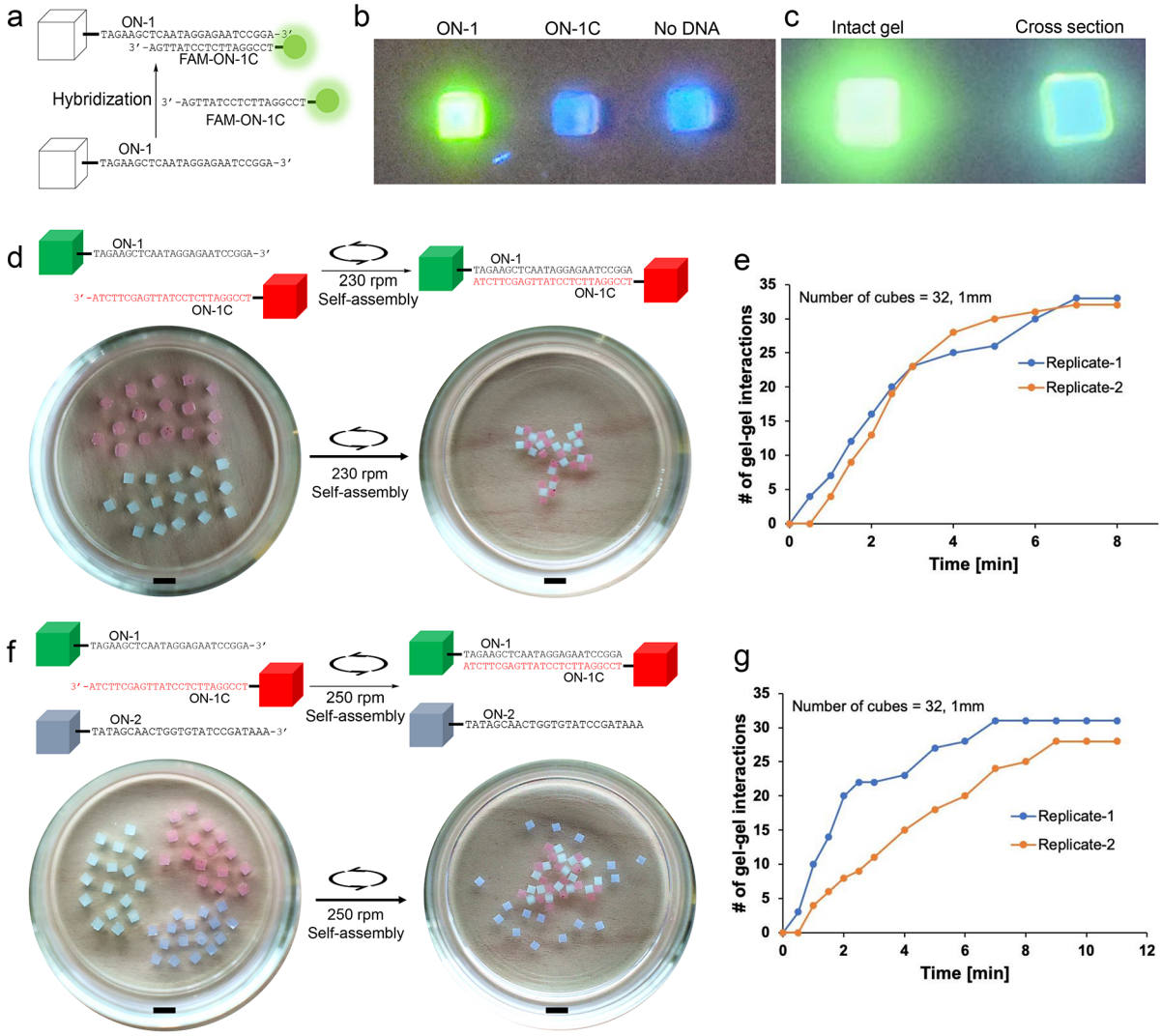

Figure 2. Characterization and self-assembly of DNA-conjugated hydrogels. (a) Procedure for quantification of the immobilized oligo DNAs. FAM-ON-1C was allowed to hybridize with ON-1 immobilized on the hydrogel. (b) Fluorescence images of FAM-ON-1C captured on hydrogels. The gels (reacted with 2 nmol of DNA) were irradiated with 365 nm light. Left: ON-1 immobilized, middle: ON-1C immobilized, right: no immobilized DNA. (c) Left: intact fluorescent gel, right: sliced hydrogel. (d) Self-assembly of 1 × 1 × 1 mm hydrogel cubes decorated with ON-1 (green) and ON-1C (red). The gels were placed in 1.2 mL of assembly buffer and agitated at 230 rpm. See Movie S1 for the entire time course. Scale bar, 3 mm. (e) Number of observed gel−gel interactions at different time points were counted from Movie S1 and its replicate (1 mm cubes decorated with ON-1/ON-1C, 16 cubes each). (f) Presence of hydrogels decorated with ON-2 (gray), which is not complementary to ON-1 or ON-1C does not affect self-assembly of the hydrogels decorated with ON-1 and ON1-C. The gels were placed in 1.2 mL of assembly buffer and agitated at 250 rpm. See Movie S2 for the entire time course. Scale bar, 3 mm. (g) Number of observed gel−gel interactions at different time points were counted from Movie S2 and its replicate (1 mm cubes decorated with ON-1/ON-1C/ON-2, 16 cubes each).

lack of contact with a complementary hydrogel during the experimental period. Nonspecific assembly between non-complementary cubes was not observed. Taken together, 1 mm cubic hydrogels decorated with complementary 25-mer DNAs specifically self-assemble at macroscopic scale under the experimental conditions.

We next explored the size limit of macroscopic self-assembly driven by DNA hybridization. We prepared ON-1 and ON-1C cubic gels with 1.5 and 2 mm edges as described above. Agitation of the hydrogels resulted in complete assembly of the 1.5 mm cubes (Figure 4a,b and Movie S4), whereas 2 mm cubes resulted in only partial assembly after 20 min (Figure S7). While observing the assembly process of the 2 mm cubic gels, we noticed that occasional vertical tumbling of the large cubic gels due to agitation may hinder assembly. We therefore produced 2 × 2 × 1.5 mm cuboids to restrict the hydrogels to lateral movements during agitation. Strikingly, the cuboid gels self-assembled within 10 min (Figure 4c,d and Movie S5). Since the volume and weight of the 2 mm cubic gels are 8× that of the 1 mm gels, it can be speculated that the strength of the gel−gel interaction needed to maintain assembly increases nonlinearly as the gel dimension increases. Whether reliable self-assembly of larger gels can be achieved using stronger DNA hybridization (e.g., longer DNAs or higher G/C content) is a subject of future investigations.

Specific Disassembly by Strand Displacement. Dynamic regulation of macroscopic self-assembly in response to various chemical and physical stimuli has been demonstrated

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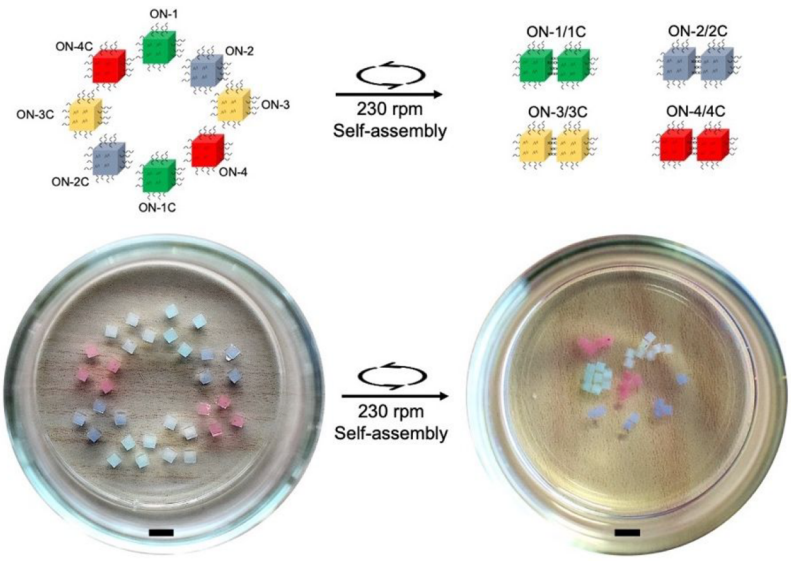

Figure 3. Self-sorting of four pairs of DNA-decorated hydrogels with complementary sequences. Four 1 mm cubes each of the hydrogels decorated with ON-1, ON-1C (green), ON2, ON-2C (gray), ON-3, ON-3C (yellow), ON-4, and ON-4C (red) were prepared and agitated at 230 rpm in a 30 mm dish containing 1.2 mL of assembly buffer. See Movie S3 for the entire time course. Scale bar, 3 mm.

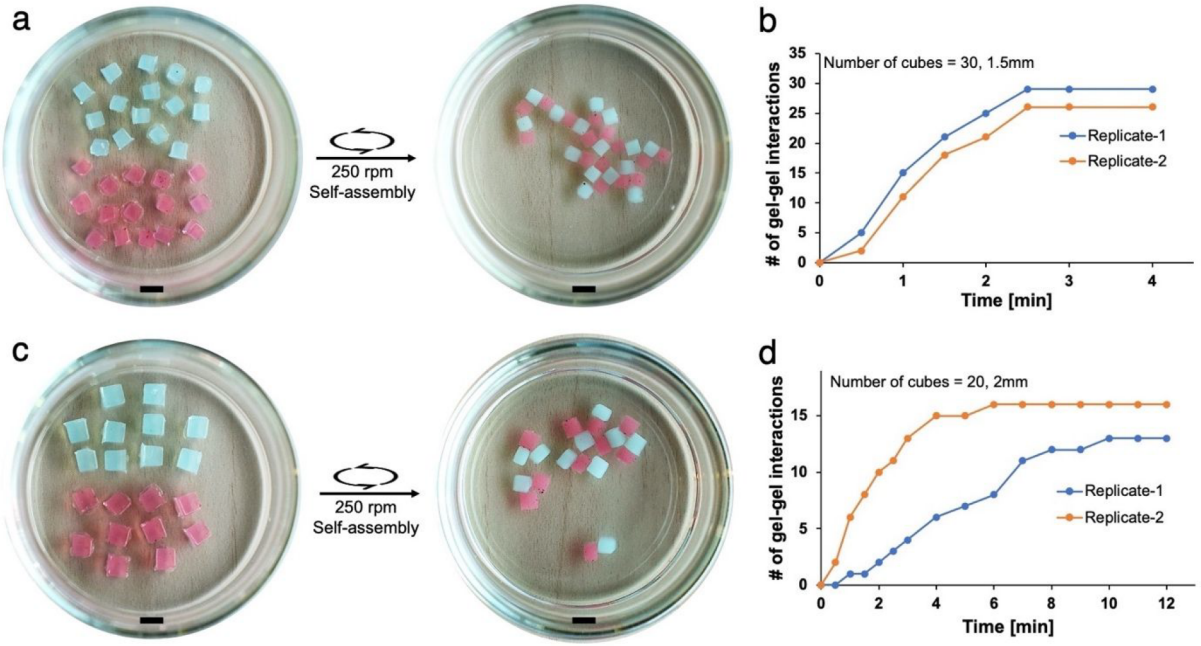

Figure 4. Self-assembly of larger gels. The gels were agitated at 250 rpm in 1.5 mL of assembly buffer in a 30 mm dish. (a) Self-assembly of 1.5 × 1.5 × 1.5 mm hydrogel cubes conjugated with ON-1 (green) and ON-1C (red). See Movie S4 for the entire time course. Scale bar, 3 mm. (b) Number of observed gel−gel interactions at different time points were counted from Movie S4 and its replicate (1.5 mm cubes decorated with ON-1/ON-1C, 15 cubes each). (c) Self-assembly of 2 × 2 × 1.5 mm cuboids carrying ON-1 (green) and ON-1C (red). See Movie S5 for the entire time-course. Scale bar, 3 mm. (d) Number of observed gel−gel interactions at different time points were counted from Movie S5 and its replicate (2 × 2 × 1.5 mm cuboids decorated with ON-1/ON-1C, 10 cuboids each).

using small molecules,[24,33] salt,[23] pH,[33,34] redox state,[22] temperature,[35] and light.[36] DNA-driven macroscopic assembly opens the possibility to control the formation or destruction of the macroscopic structures by arbitrary DNA sequences with unprecedented selectivity. To demonstrate this possibility, we sought to specifically disrupt hydrogel assemblies by DNA strand displacement. To this end, we designed ON-5 (Table S1), a 35-mer DNA that contains a 25 nt complementary sequence to ON-2C at its 5′-end. The remaining 10-nt at the 3′-end stays single-stranded and serves as a toehold to initiate strand displacement (Figure 5a). Hydrogel cubes (1 mm) decorated with ON-5 and ON-2C (red) as well as ON-4 and ON-4C (green) were prepared as described above (Figure 5a). As expected, these gels self-assembled into separate aggregates within ∼10 min (Figure 5b). Addition of ON-5C (35-mer, Table S1), which is complementary to ON-5, at a final concentration of 100 μM resulted in slow but specific disassembly of the ON-5/ON-2C gels over a period of 1 h,

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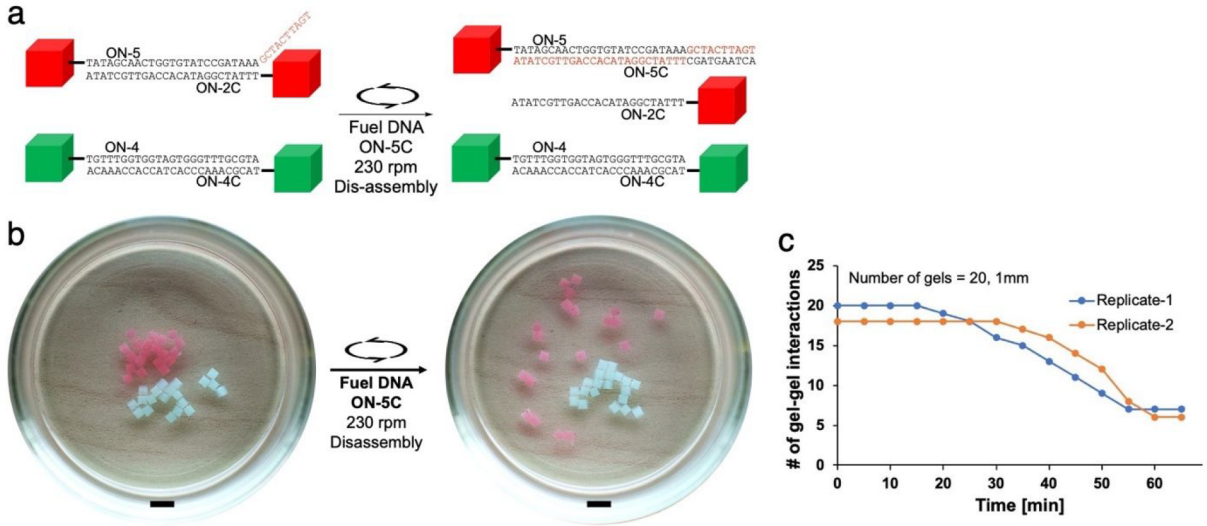

Figure 5. Specific disassembly by DNA strand displacement. (a) Schematic illustration of the DNA-decorated hydrogels (ON-5, ON-2C, ON-4, and ON-4C) and the fuel DNA (ON-5C) used in this experiment. ON-5C is expected to displace ON-2C via strand displacement due to the formation of a 35 bp duplex (ON-5/ON-5C). (b) Total of 10 hydrogel cubes (1 × 1 × 1 mm) each conjugated to ON-5, ON-2C (red), ON-4, and ON-4C (green) were first agitated at 230 rpm in 1.1 mL of assembly buffer in a 30 mm dish to form two groups of gel aggregates (left). Addition of the fuel DNA ON-5C resulted in disassembly of the red hydrogels, while the green hydrogels remained intact (right). Scale bar, 3 mm. (c) Number of gel−gel interactions at different time points were counted from video recordings (1 mm cubes decorated with ON-5/ON-2C, 10 cubes each).

while the assembled ON-4/ON-4C hydrogels remained intact (Figure 5b). The slow disassembly process (Figure 5c) may be due to the slow diffusion of ON-5C into the interface of the assembled hydrogel cubes.

■ DISCUSSION

Despite the remarkable successes of DNA-directed self-assembly at the nanometer to micrometer scale and formation of bulk gels, DNA-driven self-assembly of millimeter-sized objects has not been well-established. Nakahata and colleagues immobilized a 16-mer DNA in polyacrylamide-based gels by using an acrydite-modified oligo DNA as a monomer during polymerization.[26] They demonstrated that two hydrogels containing complementary oligo DNAs (with an 11 bp complementary sequence) adhered to each other when they were manually brought into contact. However, the researchers did not demonstrate spontaneous self-assembly of the DNA-decorated gels as they did with other systems.

In 2013, Qi et al. reported the first and only macroscopic self-assembly of DNA-modified hydrogels of up to 1 mm in size.[37] In this work, the researchers also used an acrylate-modified oligo DNA monomer for photoinitiated polymerization. However, they observed that the oligo DNAs with 20 nt complementary sequences failed to assemble the hydrogel cubes with 250 μm edges. Therefore, Qi et al. used a small circular DNA that contains a complementary sequence to the immobilized oligo DNA as a template to extend the immobilized oligo DNA through an enzymatic rolling circle amplification (RCA) reaction. This resulted in long repeat DNA that they called "giant DNA". It was shown that these giant-DNA-coated hydrogels containing complementary sequences self-assembled when agitated in a buffer solution. Although most of the experiments were performed using gels that are 200−300 μm in size, hydrogel cubes of up to 1 mm in size were shown to assemble. However, the specificity of self-assembly was moderate. For example, they reported "over 70% specific binding" of two gels (250 μm) with complementary giant DNAs. They also noted that strong agitation was necessary to disrupt nonspecific interactions, suggesting that surface-amplified DNA may also increase nonspecific interactions. Although direct comparison is not possible due to the different experimental strategies, self-assembly of our oligo DNA-decorated hydrogels was highly specific (near 100%), with no interactions observed between noncomplementary sequences. Moreover, the gels decorated with giant DNAs assembled slowly (up to hours), whereas our gels assembled within several minutes (Figure S8). Our gels are also larger (≥1 mm) compared to those used by Qi et al.[37]

A notable difference between the gel preparation protocols is that we employed postpolymerization modification by DNA, whereas Nakahata et al.[26] and Qi et al.[37] incorporated DNA during polymerization. Our strategy requires lower amounts of chemically modified DNA compared to copolymerization of DNA monomers that would inevitably trap a significant fraction of the DNA monomer in the inaccessible parts of the hydrogels. We showed that 0.88 nmol of accessible oligo DNA was immobilized on a 1.5 mm cubic gel using 2.0 nmol of the modified DNA (Figures 2b and S1). As expected, the immobilized DNAs were mostly localized on the surface (Figure 2c) making them accessible for gel−gel interaction. Although it is not clear how much oligo DNAs were incorporated in the gels produced by Qi et al., a low amount of surface-accessible DNAs may be a reason why they failed to observe self-assembly of the gels without DNA amplification.

Sequence specificity, a hallmark of DNA hybridization, was unambiguously demonstrated in multiple experiments (Figures 2f, 3, 5b). Hydrogels as large as 2 × 2 × 1.5 mm cuboids showed robust self-assembly (Figure 4c), while 2 mm cubes showed partial assembly after 20 min (Figure S7). Whether longer DNAs, lower temperature, or other modifications can extend the size of the self-assembling building blocks remain subjects of future investigations. As was observed by Qi et al., however, such efforts to strengthen gel−gel interaction may

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need to be carefully assessed against stronger nonspecific interactions.

Finally, we demonstrated dynamic regulation of DNA-directed macroscopic self-assembly by a strand displacement reaction. Strand displacement has been extensively exploited to dynamically control various DNA systems.[18] In this case, we used a fuel DNA to displace the 25 bp duplex that assembled two gels by a 35 bp duplex between the fuel strand and one of the oligo DNAs, resulting in dissociation of the two gels (Figure 5b). Such dynamic regulation of macroscopic assembly may be challenging by the giant DNA strategy[37] due to the difficulty of controlling the strength of DNA hybridization. The long time (1 h) required to disassemble the gels may be due to the slow diffusion of the fuel strand into the gel−gel interface. This limitation may be addressed by adjusting the polymer composition (e.g., lower cross-links) or using a higher temperature. Alternatively, use of a small molecule and its DNA aptamer may allow chemical regulation of gel assembly as was shown in controlling bulk DNA hydrogels.[38]

CONCLUSIONS

In summary, we described a straightforward strategy to produce millimeter-scale hydrogels that self-assemble based on short oligo DNA hybridization. This fills an important gap in the versatile repertoire of DNA-programmed self-assembly across scale and materials. Clearly, there are numerous possibilities to exploit various functions of DNAs such as molecular recognition by aptamers,[39] information processing by DNA computation,[40] and chemical catalysis by DNA,[41] to build macroscopic self-assembling systems with complex functions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.1c10308.

All experimental details including hydrogel fabrication, self-assembly, and characterization of DNA (PDF)

Movie S1. Self-assembly of 1 mm hydrogel cubes decorated with ON-1 and ON-1C. The same experiment as shown in Figure 2d (MOV)

Movie S2. Self-assembly of 1 mm hydrogel cubes decorated with ON-1, ON-1C, and ON-2. The same experiment as shown in Figure 2f (MOV)

Movie S3. Self-assembly of 1 mm hydrogel cubes decorated with ON-1, ON-1C, ON2, ON-2C, ON-3, ON-3C, ON-4, and ON-4C. The same experiment as shown in Figure 3 (MOV)

Movie S4. Self-assembly of 1.5 mm hydrogel cubes decorated with ON-1 and ON-1C. The same experiment as shown in Figure 4a (MOV)

Movie S5. Self-assembly of 2 × 2 × 1.5 mm hydrogel cuboids decorated with ON-1 and ON-1C. The same experiment as shown in Figure 4c (MOV)

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Notes

The authors declare no competing financial interest.

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