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Supporting Information

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Multicomponent DNA Polymerization Motor Gels

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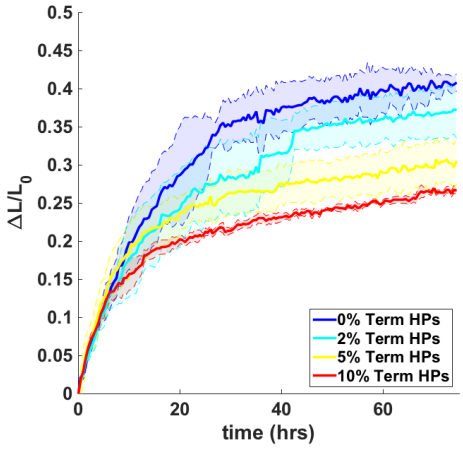

Figure S1. Graph showing the linear swelling ratio as a function of time for the PEGDA10kMW-DNA gel swelling in 20μM DNA hairpins with 0, 2, 5, and 10% of terminator DNA hairpin monomers.

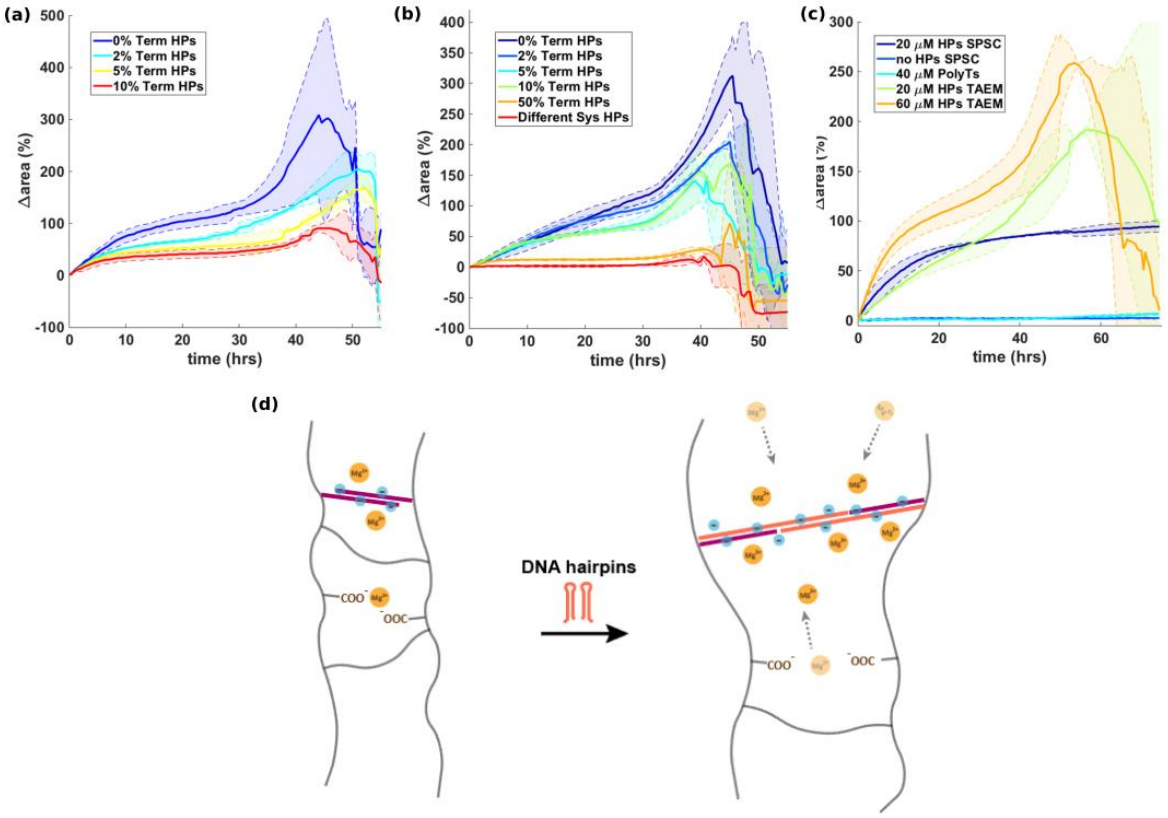

Figure S2. Graphs of the area swelling ratio vs. time for GelMA-DNA gels. We utilized the swelling ratio Δarea (change in the area with respect to the original area) instead of $\Delta L/L_0$ (change in the side length with respect to the original side length) because the GelMA-DNA gel is very soft and the corners often appear blurred, especially when the gel is dissolving. Hence, there is more variance in length than in area.) a) GelMA40%DoS-DNA swelling in 20μM DNA hairpins with TAE/Mg^{2+} (TAEM) buffer with 0, 2, 5, and 10% of terminator DNA monomers. b) GelMA80%DoS-DNA swelling in 20μM DNA hairpins with TAEM with 0, 2, 5, 10, and 50% of terminator DNA monomers and HPs from a different system (system1 HPs as in Table S1). c) GelMA40%DoS-DNA gel swelling with HPs in SPSC and 20 nt PolyT DNA strands in TAEM, compared with samples swelling with TAEM buffer. d) Schematic of hypothesized Mg^{2+} ion competitive binding in GelMA-DNA.

We hypothesized that the dissolution of GelMA-DNA hydrogels at ~50hrs after the addition of DNA hairpins was due to a high degree of DNA-directed swelling. To vary the extent of swelling, we used different fractions of terminator hairpins, since high proportions of these terminator hairpins should limit crosslink chain growth, and thus the extent of swelling.[1] For example, a hairpin solution containing 50% terminators should allow, on average, the incorporation of only two hairpins per hydrogel chain. While the swollen sizes after 40 hours of the hydrogels stimulated with hairpin mixtures containing different fractions of terminations were different, the hydrogels all disintegrated around 50 hr (Figure S2a). These results suggested that the growth of long crosslink chains was not the cause of the hydrogels' dissolution.

Due to the chemical crosslink generated between methacrylate groups through free radical polymerization, the degree of methacrylation ratio will affect the crosslink density and mechanical properties.[2] As a result, we first speculated that the destruction of the GelMA-DNA hydrogels was due to the low cross-linking density of the 40% methacrylation ratio gelatin pre-polymer. To test this hypothesis, we swelled 80% degree of substitution GelMA-DNA hydrogels using DNA hairpins. We found, however, that these hydrogels also melted after being triggered with DNA hairpins across the same range of terminator hairpin fractions (Figure S2b).

We next tested the response of the GelMA-DNA gels to DNA hairpins with sequences that were not complementary to the DNA crosslinks in the hydrogel. Adding these hairpins would be expected to cause no hydrogel size change, as has been observed previously for Am-DNA gels.[1] There was no change in hydrogel size for about 40-50 hours after which time the gels dissolved (Figure S2b). These results suggested that dynamic interaction of species that can interact both with the hydrogel network and with free DNA hairpins in solution, rather than the interaction of the DNA hairpin strands with the hydrogel crosslinks, was the cause of the GelMA-DNA gel dissolution.

It has been reported that photo-crosslinked GelMA hydrogels of 5 wt% (without DNA crosslinks) are degradable and can dissolve in a collagenase solution in less than three days. This result also indicates the dissolution could happen with GelMA hydrogel.[3] So we looked more into the other contents of the swelling solution that may react with the GelMA backbone. TAE/Mg^{2+} buffer has been widely used in the study of the DNA hybridization chain reaction used to direct swelling within polymerization motor gels; the magnesium ions in the buffer help screen the negative charges on the DNA phosphate backbone and stabilize hybridized DNA.[4] However, the ionized carboxylic acid groups of the gelatin backbone, which carry negative charges and can also bind with the Mg^{2+} and form ionic crosslinks alongside the C-C bonds.[5] After the DNA hairpins are added, the Mg^{2+} that originally bonded to the gelatin polymer chain could transfer to the DNA phosphate backbone that has a higher charge density, thus breaking the ionic crosslinks and causing the dissolution of GelMA-DNA hydrogel.

Researchers also found the divalent ions decreases the stability of the gelatin hydrogel in that it will bind with the carboxylic acid groups in the polypeptide and weakening the electrostatic interactions between the carboxylic acid groups and amine groups on GelMA chain.[5] The effect of Mg^{2+} itself on the degradation of GelMA hydrogel might not be enough for complete dissolution, which also agrees with the fact that our GelMA-DNA gel remain intact within Mg^{2+} buffer for weeks. However, when DNA hairpins are also presented with Mg^{2+}, ion transfer between GelMA chain as ionic crosslinks and DNA phosphate backbone can significantly speed up the dissolution process. It also gave us hint in using Na^{+} as an alternative for Mg^{2+}, as 1) it would lead to a more stable gel with more interacted carboxylic acid groups and amine groups; 2) since monovalent ion cannot form ionic crosslinks between two polymer chains, it would prevent ion transfer disrupting the gel structure.

We note that it is conceivable that increasing the Mg^{2+} concentration to saturate the polymer network might also work, but the Mg^{2+} concentration in TAEM (12.5mM) is already much higher than the concentration in human plasma and DMEM (below 1mM) and further

increasing it could affect cell viability and functionality as Mg^{2+} is an essential mineral implicated in many cellular functions and acts as a cofactor for several enzymes.[6] Instead by using an alternate ion such as Na^{+}, we were able to use reasonable concentrations which as demonstrated allow cell viability and this is a highlight of the current approach.

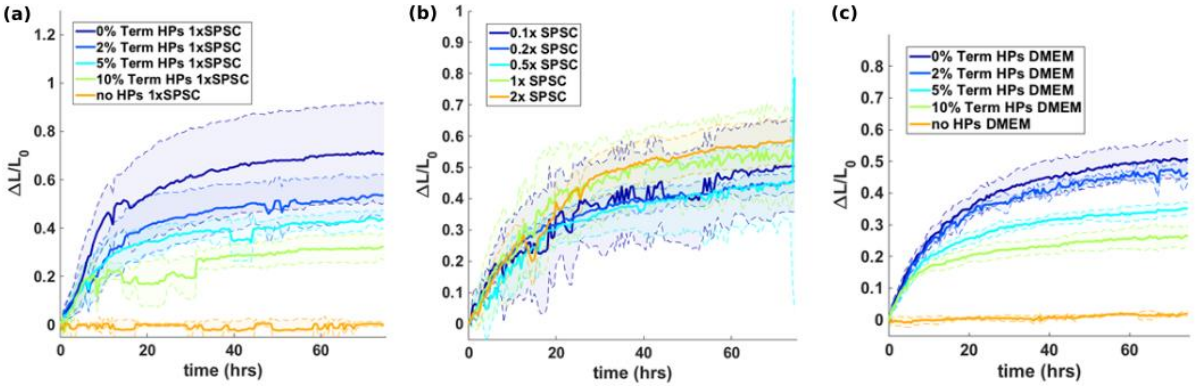

Figure S3. a) The GelMA80%DoS -DNA gel swelling profile in Na^{+} (1xSPSC) buffer with 0, 2, 5, and 10% of terminator DNA monomers. b) The GelMA80%DoS-DNA gel swelling profile in different concentrations of sodium buffer (SPSC). c) The GelMA80%DoS-DNA gel swelling profile in DMEM (sodium concentration ~120mM) with 0, 2, 5, and 10% of terminator DNA monomers.

The sodium ion concentration in 1x SPSC buffer is approximately 1M, which is unsuitable for cell culture applications. We thus investigated the swelling characteristics under lower salinity. The results showed that the salinity could affect the swelling ratio by about 30%; however, even under 0.1x SPSC, where the Na^{+} concentration is ~100mM, the DNA polymerization process could still proceed. Then the swelling experiments were conducted in cell culture media DMEM with no additional salt added, and the results indicated the GelMA-DNA hydrogel could achieve controllable swelling with the DMEM prepared DNA hairpin solution.

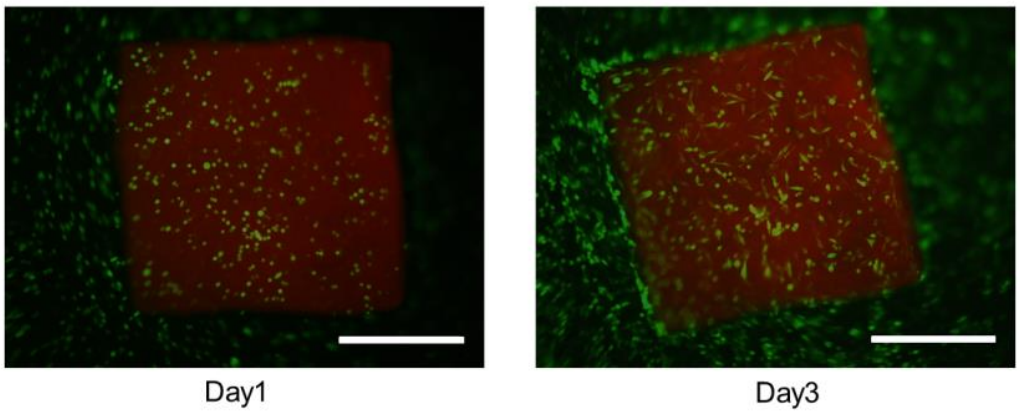

Figure S4. Hela/GFP reporter cell(green) culture on GelMA80%DoS-DNA hydrogel(red) without DNA directed swelling. The scale bar represents 500μm. The gel was not disturbed after seeding and during cell culture. Thus, the cells grew on top and around the hydrogel. It has been reported that cell confluency for GelMA hydrogels depends on the wt% of the pre-polymer, and our result here was similar to prior results using pure GelMA hydrogel with the same 5 wt% of GelMA.[7]

Quantifying the extent of swelling of the hydrogel using imaging and MATLAB scripts. Either the side-length or area of the 2D projection of the hydrogel was calculated using custom MATLAB scripts developed using edge-detection algorithms and used to measure the extent of swelling. The algorithm utilized thresholding to determine the boundaries of the square-shaped hydrogel. The side length of the hydrogel was determined by calculating the mean value of the distances between the four vertices of the square hydrogel. Details of the threshold value calculation and vertices searching method were described in *Fern et al*.[8] The code is available upon request.

DNA/PEG concentration ratio calculation. As described in the main text, we used a 10 wt% of PEGDA pre-polymer for the PEGDA 575MW-DNA and PEGDA 10kMW-DNA hydrogels. The corresponding molar concentration of PEGDA 575MW and PEGDA 10kMW was 0.174M and 0.01M, respectively. The DNA crosslinking duplex concentration was 1.154mM. As the DNA crosslinking duplex and the PEGDA pre-polymer have two reaction sites to generate crosslinks through free radical polymerization, we can estimate that the DNA/PEG concentration ratio is 0.66% (1.154mM/174mM) for PEGDA 575MW-DNA and 11.54% (1.154mM/10mM) for PEGDA 10kMW-DNA.

Table S1. DNA strands used

DNA strand	Sequence
S2-C	/5Acryd/CTGTCTGCCTACCACTCCGTTGCG
S2-C’	/5Acryd/ATTCGCAACGGAGTGGTAGGCTTT
S2-H1	AAAGCCTACCACTCCGTTGCGGAACCTCG CAACGGAGTGGTAGGCAGACAG
S2-H2	AGGTTCCGCAACGGAGTGGTAGGCCTGTC TGCCTACCACTCCGTTGCGAAT
S2-H1T	AAAGCCTACCACTCCGTTGCGTCAAGCCG CAACGGAGTGGTAGGCAGACAG
S2-H2T	AGGTTCCGCAACGGAGTGGTAGGCAATCG TGCCTACCACTCCGTTGCGAAT
S1-H1(control hairpin)	CCACGCTGTGGCACCTGCACGCACCCACG TGCAGGTGCCACAGCGAACTTA
S1-H2(control hairpin)	TGGGTGCGTGCAGGTGCCACAGCGTAAGT TCGCTGTGGCACCTGCACGTTG
PolyTs(20 bases)	TTTTTTTTTTTTTTTTTTTT

Table S2. Shear modulus (G' and G") test results

Hydrogel type	As prepared (after hydration) G'/G"(Pa)	1day DNA swollen G'/G" (Pa)	2 days DNA swollen G'/G" (Pa)
Am-BIS(19:1)	758.6±38.3 53.8±33.5		
Am-DNA	491.2±17.1 50.4±16.7	307.3±52.6 35.1±25.2	229.5±20.0 31.4±4.8
Am-2mMBIS	553.8±20.4 27.7±4.8		
Am-2mMBIS-DNA	662.5±88.4 71.0±30.8	534.0±33.2 45.3±6.4	440.0±90.1 33.1±20.4
Am-5mMBIS	981.5±168.8 59.7±28.8		
Am-5mMBIS-DNA	1209.3±71.6 75.0±33.8	647.2±80.7 58.5±12.0	522.4±100.9 30.3±8.2
Am-10mMBIS	1698.6±108.9 134.4±97.2		
Am-10mMBIS-DNA	2255.1±137.3 147.3±8.2	933.3±246.1 55.8±4.1	810.7±28.8 69.7±3.3
PEGDA10kMW	2691.1±93.5 183.3±25.8		
PEGDA10kMW-DNA	3887.8±493.4 178.3±95.2	2168.3±217.2 136.1±78.4	1761.9±218.5 112.0±30.8
GelMA80%DoS	231.4±64.2 20.9±10.8		
GelMA80%DoS-DNA	297.0±23.0 28.1±21.4	245.9±8.3 54.1±9.0	177.9±14.8 42.6±4.8

Captions for Movies

Movie S1. Time-lapse fluorescence video of the Am-2mMBIS-DNA hydrogel swelling in 20 μM DNA hairpin solution (TAE/12.5mM Mg^{2+}, 98% polymerizing hairpin and 2% terminator hairpin).

Movie S2. Time-lapse fluorescence video of the PEGDA20kMW-DNA hydrogel swelling in 20 μM DNA hairpin solution (TAE/12.5mM Mg^{2+}, 98% polymerizing hairpin and 2% terminator hairpin).

Movie S3. Time-lapse fluorescence video of the GelMA-DNA hydrogel swelling in 20 μM DNA hairpin solution (1x SPSC buffer, 98% polymerizing hairpin and 2% terminator hairpin). The GelMA pre-polymer has an 80% methacrylate group substitution ratio.

Movie S4. Time-lapse fluorescence video of the GelMA-DNA hydrogel swelling and disassembling in 20 μM DNA hairpin solution (TAE/12.5mM Mg^{2+}, 98% polymerizing hairpin and 2% terminator hairpin). The GelMA pre-polymer has an 80% methacrylate group substitution ratio.

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