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# Multicomponent DNA Polymerization Motor Gels

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**Hydrogels with the ability to change shape in response to biochemical stimuli are important for biosensing, smart medicine, drug delivery, and soft robotics. Here, a family of multicomponent DNA polymerization motor gels with different polymer backbones is created, including acrylamide-*co*-bis-acrylamide (Am-BIS), poly(ethylene glycol) diacrylate (PEGDA), and gelatin-methacryloyl (GelMA) that swell extensively in response to specific DNA sequences. A common mechanism, a polymerization motor that induces swelling is driven by a cascade of DNA hairpin insertions into hydrogel crosslinks. These multicomponent hydrogels can be photopatterned into distinct shapes, have a broad range of mechanical properties, including tunable shear moduli between 297 and 3888 Pa and enhanced biocompatibility. Human cells adhere to the GelMA-DNA gels and remain viable during ≈70% volumetric swelling of the gel scaffold induced by DNA sequences. The results demonstrate the generality of sequential DNA hairpin insertion as a mechanism for inducing shape change in multicomponent hydrogels, suggesting widespread applicability of polymerization motor gels in biomaterials science and engineering.**

## 1. Introduction

Hydrogels have been widely investigated as important functional materials due to their similarity to biological tissue.[1] The high water content, low interfacial tension, and high permeability provide unique environments of relevance to drug delivery, tissue engineering, and soft robotics.[1,2] Recently, self-folding, shape change, and 4D printed devices have been developed using stimuli-responsive hydrogels toward the ultimate goal of creating biomimetic, autonomous, and adaptive smart structures, robots, and biomedical devices.[3] Among the different stimuli-responsive hydrogels, biomolecules can be used to induce shape change without the physiological side effects that may occur in response to traditional shape change stimuli, such as temperature, pH, light, ultrasound, or electric field.[4] Hydrogels that respond to specific DNA sequences are also important because they can be structured in a combinatorial variety of ways to program responsivity to different biomolecular stimuli.[5] Such biomaterials could potentially detect physiological signals and direct therapeutic release or apply mechanical forces to control tissue formation or remodeling.[6]

Recently, we described acrylamide (Am)-co-DNA hydrogels in which sequential insertion of DNA hairpins with specific sequences induces high-degree expansion of the hydrogels.[7] In contrast to conventional DNA stimuli-responsive gels that swell uniaxially by only 10–20%, these DNA polymerization motor gels can swell significantly, by over 100-fold volumetrically.[7,8] The term "motor" here refers to the chemical cascade that occurs at the hydrogel crosslinks upon stimulation by the DNA sequence input.[7,9] The swelling process is driven by the operation of a polymerization motor that operates by processively incorporating DNA sequence inputs, i.e., fuel, into the hydrogel's crosslinks, lengthening them.[7,9,10] Such insertion polymerization at a specific initiation site can perform work by displacing structures at the ends of the polymer, as actin polymerization displaces a membrane.[11] In this context, this polymerization, which displaces the copolymers at the crosslinks' ends and leads to DNA accumulation within the hydrogel, drives hydrogel swelling.[7] These high-swelling DNA polymerization motor gels can deliver high strains and cause significant bending key for many potential applications in biology and medicine.[12] The DNA crosslinks can also be modified to lock and unlock DNA hairpin-induced swelling by either DNA sequences or other chemical signals, which can also be amplified before swelling.[13] DNA-directed swelling could therefore also be a platform to allow swelling in response to a variety of chemical stimuli at different concentrations or combinations of stimuli.[13]

Materials with different molecular components and physical properties such as moduli or swelling propensity are critical to

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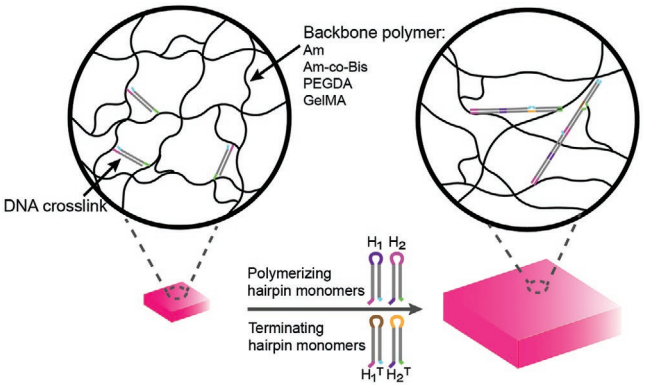


**Figure 1.** Conceptual schematic of multicomponent DNA polymerization motor gels. Gels containing DNA crosslinks with different backbone polymers: Am, Am-BIS, PEGDA, and GelMA, before (left panel) and after (right panel) swelling in an aqueous buffered solution of polymerizing and terminating DNA hairpins.

embody different functionalities and applications, as is evident by observing the diversity of soft tissues in the human body (e.g., brain vs muscle) and animals (e.g., jellyfish vs elephant).[14] One of the limitations of our previously described DNA polymerization motor gels is that they were based on an acrylamide copolymer which lacked covalent chemical crosslinks, resulting in very soft (e.g., shear modulus about 500 Pa) hydrogels. The ultrasoft nature of this DNA polymerization motor gel makes it challenging to handle, process, and integrate into functional devices as they are fragile and prone to tearing. Also, the previously utilized uncrosslinked Am monomer residue is potentially a carcinogen and can cause peripheral neuropathy, which can limit the use of this DNA polymerization motor gels in biomedical applications in vivo.[15]

In this paper, we investigate the development of DNA polymerization motor gels with chemical crosslinks that give them a Young's modulus up to an order of magnitude larger than our previously reported DNA polymerization gels. We also study the possibility of creating DNA polymerization motor gels with biocompatible copolymers for potential tissue engineering applications. Specifically, we investigate the creation of DNA polymerization motor gels with the following components: acrylamide (Am), acrylamide-*co*-*N*, *N*′-methylenebis (acrylamide) (Am-*co*-BIS, or Am-BIS), polyethylene glycol diacrylate (PEGDA) and gelatin-methacryloyl (GelMA) (**Figure 1**). The choice of each of these polymers establishes how a variety of polymer backbones with different chemical properties and affinities for hydration can be driven to swell by DNA strand displacement. Am has a high affinity for water, and we utilized it in our previous work; in this study, it serves as the control sample.[7,16] By adding *N*,*N*′-methylenebis (acrylamide), (BIS), a commonly used crosslinker for hydrogels, we investigated how the storage modulus of the Am-DNA material can be tuned and increased significantly.[17] We studied the inclusion of polyethylene glycol (PEG) as a component in our DNA gels because of good biocompatibility, high oxygen permeability, and relatively robust mechanical properties.[1a,18] We developed gelatin-based DNA polymerization motor hydrogels since gelatin is an irreversibly hydrolyzed form of collagen with good biocompatibility, cell adhesion, and biodegradation properties, and has previously been used to create cross-linked matrices to study the behavior of cells in synthetic environments and is widely utilized in cell culture and tissue engineering.[19] These new multicomponent materials demonstrate how the DNA polymerization motor provides a general means to induce a stimuli-responsive swelling behavior in hydrogels and open up new opportunities to use DNA sequences or other biomolecules to induce hydrogel shape change.

## 2. Results and Discussion

### 2.1. Acrylamide-*co*-(Bis-Acrylamide)-*co*-DNA (Am-BIS-DNA)

Polyacrylamide (PAAm) gels composed of the Am monomer are highly water absorbent, forming a soft gel when hydrated and used in biochemistry and bioanalytical chemistry.[16,20] BIS is a bi-functional molecule commonly used to cross-link acrylamide via a free radical vinyl polymerization mechanism.[21] By varying the concentration of BIS, it is possible to tune the pore size and stiffness of PAAm gels.[17,21,22]

We created Am-BIS-DNA hydrogels by cross-linking Am (10 wt%) and Acrydite modified DNA duplex crosslinker ($1.154 \times 10^{-3}$ M) with 2, 5, or $10 \times 10^{-3}$ M of BIS. We photopatterned 1 mm × 1 mm × 160 µm square shaped gels using a previously described protocol.[7,18c,23] We observed that the Am-BIS-DNA hydrogels formed were less sticky, easier to handle, and less prone to rupture during processing than the Am-DNA hydrogels. We rationalize this observation by noting that since BIS is a bifunctional crosslinker, it enhances the extent of cross-linking, leading to a stiffer gel.[22,24] In contrast to Am-DNA gels, Am-BIS-DNA gels have two kinds of crosslinks, one involving the covalent C—C bonds between the cross-linked vinyl groups and the other involving hydrogen bonding between DNA nucleobases.

While polymerizing hairpins extend the crosslinks by strand displacement to cause swelling, terminator hairpins with non-complementary hairpins terminate strand displacement and arrest swelling.[7] By changing the ratio of polymerizing/terminating strands, we can tune the swelling of hydrogel to a desired final size. We noticed that the use of $20 \times 10^{-6}$ M of 98% polymerizing and 2% terminator DNA hairpin solution gave well-controlled swelling results, so we utilized the same ratio of polymerizing/terminating hairpins for all our swelling experiments throughout the paper unless otherwise stated.

Despite the existence of covalent C–C crosslinks that are up to 8.7× ($10 \times 10^{-3}/1.154 \times 10^{-3}$ M) of DNA crosslinks in the Am-BIS-DNA hydrogels, we observed that they could undergo DNA-induced expansion (**Figure 2**b). The hydrogels expanded in response to polymerizing and terminating DNA hairpins and maintained their square shape after expansion. We observed that the rate of gel swelling and the swelling ratio $\Delta L/L_0$ (change in the side length with respect to the original side length) both decreased with increasing concentration of BIS (Figure 2c). We attribute this observation of reduced swelling to the increased stiffness of this gel, which is reflected in the modulus measurements (Figure 2d). By increasing the BIS concentration, we

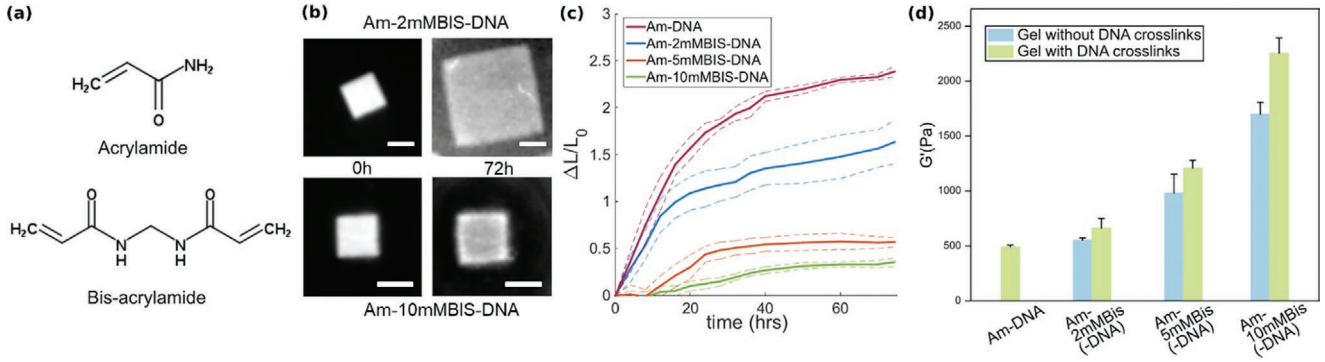


**Figure 2.** Am-BIS-DNA hydrogels. a) Molecular structures of acrylamide and bis-acrylamide. b) Fluorescence images of Am-$2 \times 10^{-3}$ M BIS-DNA and Am-$10 \times 10^{-3}$ M BIS-DNA gels immediately (0 h) after and 72 h after immersion in a buffer solution containing $20 \times 10^{-6}$ M of 98% polymerizing, 2% terminating hairpins. The scale bar represents 1 mm. c) Graphs of the linear swelling fractional ratio of square Am-BIS-DNA hydrogel films plotted as a function of time for different concentrations (0, $2 \times 10^{-3}$, $5 \times 10^{-3}$, and $10 \times 10^{-3}$ M) of BIS. The dotted lines surrounding each curve represent the standard deviations over at least three measurements. d) Bar plot of the shear moduli of Am-BIS hydrogels with different concentrations of BIS (0, $2 \times 10^{-3}$, $5 \times 10^{-3}$, and $10 \times 10^{-3}$ M) with and without DNA crosslinks.

are also increasing the non-DNA or C–C crosslink concentration; these crosslinks resist swelling of the gel that is driven by the DNA polymerization motors. It is noteworthy that Am-BIS hydrogels are stiffer with DNA than without DNA at all concentrations of BIS, which suggests that the double-strand DNA crosslinks in the gels also strengthen the gels. These results indicate that the inclusion of stimuli unresponsive chemical crosslinks (i.e., BIS) in an Am gel while stiffening the gel still allows it to swell by the DNA polymerization motor, although the extent of swelling is decreased. Together, our findings show that BIS concentration can be utilized to tailor the modulus and swelling ratio of the DNA polymerization motor gels.

### 2.2. PEGDA-*co*-DNA (PEGDA-DNA)

PEGDA is a biocompatible polyether with many applications from industrial manufacturing to medicine.[1a,18c,25] By co-polymerizing PEGDA with an Acrydite-modified DNA duplex crosslinker at both C and C′ strands, we were able to form PEGDA-DNA polymerization motor gels. We investigated the effect of PEGDA pre-polymer molecular weight on the swelling characteristics of these gels in response to polymerizing and terminator DNA hairpins, as shown in **Figure 3**b,c.

The molecular weight (MW) of the PEGDA pre-polymers strongly affects the mechanical properties of the crosslinked PEGDA hydrogels. For example, previous studies have shown that photo-crosslinked PEGDA gels with pre-polymers of 700 MW had a shear modulus of 241 kPa as compared to 4.1 kPa for a pre-polymer with 10k MW.[18c] We studied PEGDA-DNA gels composed of PEGDA 575 MW, 6k MW, 10k MW, and 20k MW with the same pre-polymer concentration (10 wt%) and Acrydite-modified DNA duplex crosslinker concentration ($1.154 \times 10^{-3}$ M). We observed a significantly faster swelling speed and larger swelling ratio $\Delta L/L_0$ for gels with a higher MW of PEGDA, consistent with the dependence of the degree and rate of swelling on hydrogel stiffness observed for Am gels. In fact, no significant swelling was observed for gels with 575 MW PEGDA, suggesting that the DNA polymerization motors used at the concentrations, and associated hydration do not apply enough force to swell relatively high shear modulus gels (based on the literature cited value of 241 kPa for 700 MW PEGDA).[18c] We note that in our experiments, the ratio of DNA crosslinks/PEG crosslinks is very small; we can estimate the ratio as 0.66% for the PEGDA575 MW-DNA and 11.54% for the PEGDA10k MW-DNA (see calculation details in the Supporting Information). This low fraction of DNA crosslinks in the 575 MW PEGDA gels should also contribute to the small extent of DNA-directed swelling observed in this gel since it is the hydrogen bond crosslinks that increase in length when DNA polymerizing hairpins are introduced into the solution.

### 2.3. GelMA-*co*-DNA (GelMA-DNA)

GelMA is the methacrylation product of gelatin, which is a denatured form of collagen.[19b,26] We investigated the creation of GelMA-DNA polymerization motor gels for potential tissue engineering applications.

First, we attempted to incorporate DNA crosslinks into the GelMA network and were able to photo-polymerize GelMA-DNA gels. During the swelling of the GelMA-DNA gels in the DNA hairpin solution, we observed that after an initial period of swelling, the swelling sped up, the gel became less detectable in fluorescence and eventually disappeared after 50 h (**Figure 4**b and Figure S2a, Movie S4, Supporting Information). We investigated the disappearance of GelMA-DNA polymerization motor gels on swelling with DNA hairpins by systematically varying the methacrylate crosslink density and terminator to polymerizing hairpin ratio. We also studied the stability of the gels in non-complementary DNA hairpins (see detailed data in the Supporting Information). We observed that the gel always disappears in the presence of DNA hairpin strands and the TAE/$Mg^{2+}$ buffer, which is commonly used in DNA technology to screen the negative charge on the phosphate backbone and facilitate DNA hybridization.[27] Surprisingly, the gels also dissolved even when immersed in a solution containing non-complementary DNA hairpins. This result suggests that the dissolution process

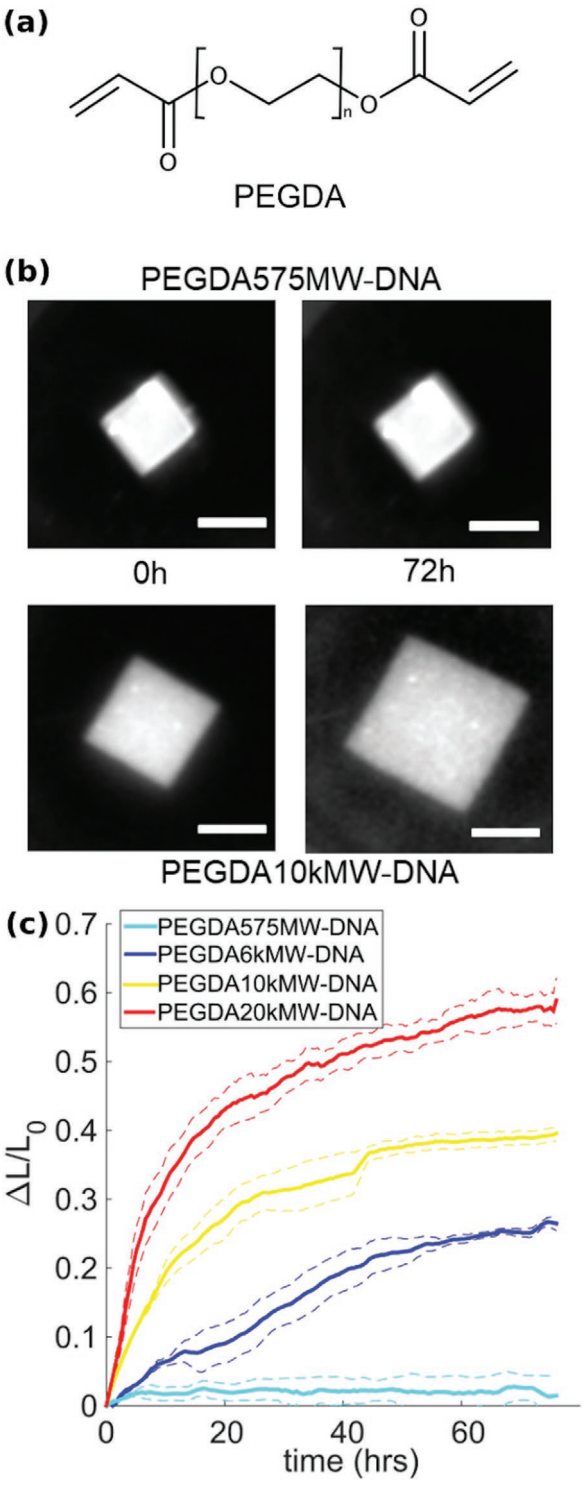


**Figure 3.** DNA directed swelling of PEGDA-DNA hydrogels. a) Molecular structure of PEGDA. b) Fluorescence images of PEGDA575 MW-DNA and PEGDA10k MW-DNA gels immediately (0 h) after and 72 h after immersion in a buffer solution containing $20 \times 10^{-6}$ M of 98% polymerizing, 2% terminating hairpins. The scale bar represents 1 mm. c) The linear swelling fractional ratio of square PEGDA-DNA hydrogel films plotted as a function of time for different PEGDA molecular weights of 575, 6k, 10k, and 20k. The dotted lines represent the standard deviation over at least three measurements.

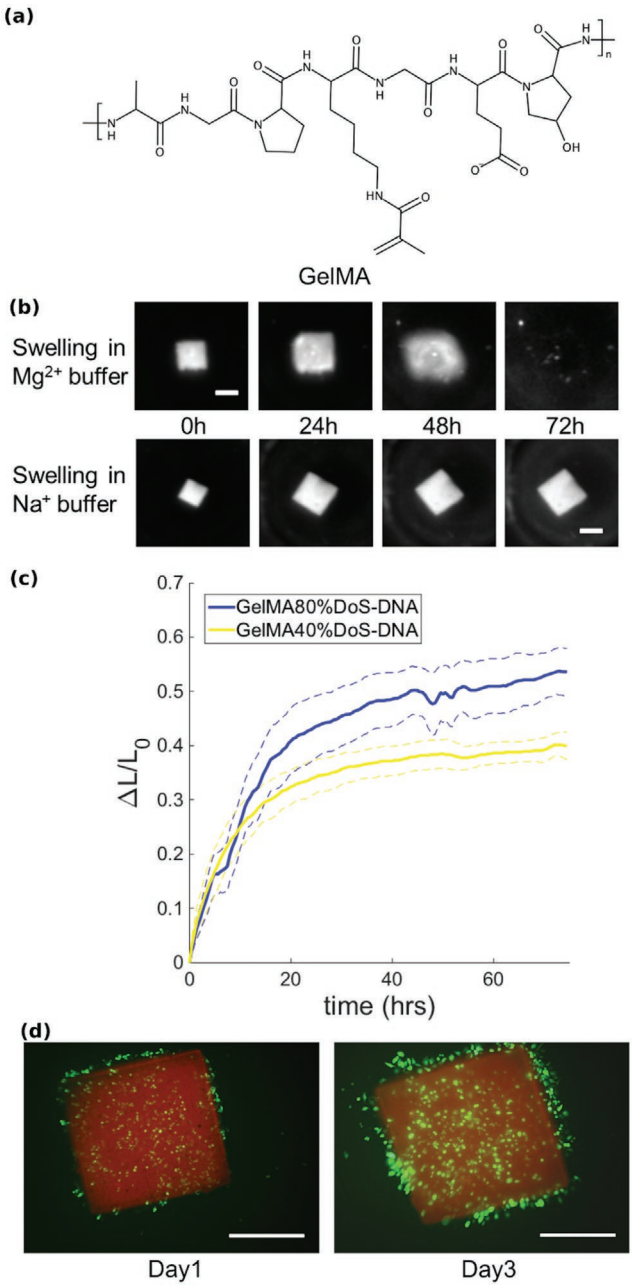


**Figure 4.** GelMA-DNA hydrogels. a) Molecular structure of GelMA. b) Fluorescence images of GelMA80% DoS-DNA gels 0, 24, 48, and 72 h after immersion in $20 \times 10^{-6}$ M of 98% polymerizing, 2% terminating DNA hairpins of $Mg^{2+}$ buffer and $Na^{+}$ buffer. The scale bar represents 1 mm. c) Graphs of the linear swelling fractional ratio versus time for square-shaped GelMA-DNA hydrogels with 40% or 80% methacrylate group DoS. d) Fluorescence images of HeLa/GFP reporter cells (green) cultured on the GelMA-DNA gels (red) on the first and third day of DNA-directed swelling. The scale bar represents 500 µm.

does not involve a direct change in the gel structure due to DNA hairpin insertion, but rather dynamic interaction of species that can interact both with the hydrogel network and with free DNA hairpins in solution. We note that the GelMA pre-polymer has ionized carboxylic acid groups, which associate with the divalent

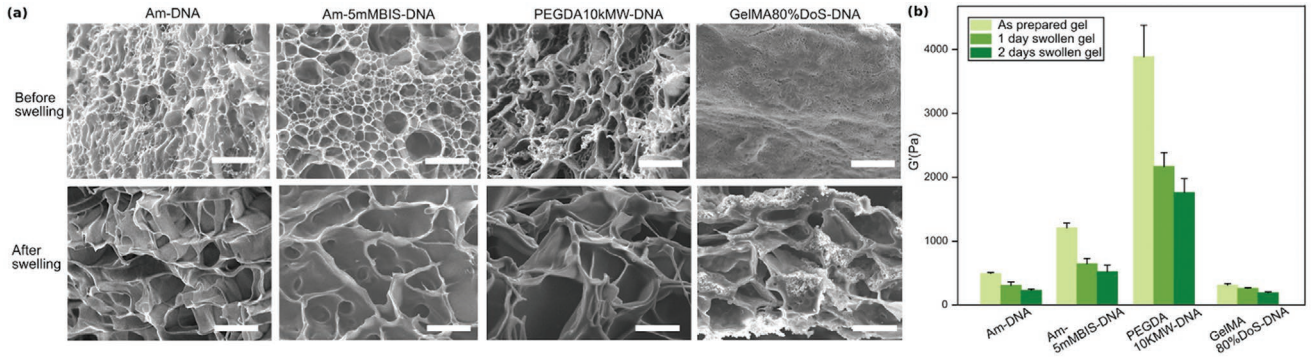


**Figure 5.** Microstructure and modulus characterization of the multicomponent DNA polymerization motor gels. a) SEM images of the Am-DNA, Am-5 × $10^{-3}$ M BIS-DNA, PEGDA10k MW-DNA, and GelMA80% DoS-DNA, as prepared and after swelling 2 days in 20 × $10^{-6}$ M of 98% polymerizing, 2% terminating DNA hairpin solution. The SEM images were taken of gels that were frozen and lyophilized. The scale bar represents 50 µm. b) Bar plot of the shear moduli of the Am-DNA, Am-5 × $10^{-3}$ M BIS-DNA, PEGDA10k MW-DNA, and GelMA80% DoS-DNA gels before and after DNA-directed swelling for 1 and 2 days. The moduli were measured at 1% strain and a frequency of 2 Hz.

$Mg^{2+}$ ions and act as a bidentate ligand to form ionic crosslinks within GelMA-DNA gel.[28] We postulated that as DNA hairpins were added, the $Mg^{2+}$ ions compete between the phosphate backbone of DNA and carboxylic acid groups in GelMA, disrupt the ionic crosslinks and destabilizing the gel. We tested this hypothesis by substituting the divalent $Mg^{2+}$ ion buffer with a buffer containing only monovalent $Na^+$ ions, sodium phosphate-sodium chloride (SPSC, 1 M NaCl, 50 × $10^{-3}$ M $Na_2HPO_4$; pH 7.5) buffer while keeping the crosslink and hairpin content the same as stated above. We observed that GelMA-DNA gels with both 40% and 80% methacrylate group degree of substitution (DoS) swelled and reached a stable final size in response to DNA hairpins in SPSC (Figure 4c). We tested whether gels would swell in a buffer with a lower sodium concentration using Dulbecco's Modified Eagle Medium (DMEM) cell culture media as a buffer ([$Na^+$] ≈120 × $10^{-3}$ M) and observed that DNA directed swelling occurred in DMEM (Figure S3c, Supporting Information).

GelMA hydrogels are widely used for cell adhesion, proliferation, and migration studies since they contain the RGD (arginine-glycine-aspartic acid) cell adhesive peptide motif.[19b] Incorporating GelMA instead of Am or PEGDA as the copolymer enhances the biocompatibility of the gel for cell culture applications without adding peptides or proteins.[29] To validate cell compatibility of GelMA-DNA gels, we cultured fluorescent HeLa GFP reporter cells and verified green fluorescence on GelMA-DNA hydrogels. Cell density will increase with increasing GelMA concentration, and despite the relatively low fractional concentration (5 wt%) of GelMA in our GelMA-DNA hydrogel, we still observed high cell confluency after 2 days in culture.[19b] Importantly, cells that were first adhered to as prepared GelMA-DNA gels were viable even after DNA directed swelling was induced by adding a DNA hairpin solution to a final concentration of 10 × $10^{-6}$ M (Figure 4d).

### 2.4. Microstructure and Modulus Measurements

To understand how the gel's mechanical properties and structure could be varied using a DNA stimulus, we characterized the microstructure of the Am-DNA, Am-5 × $10^{-3}$ M BIS-DNA, PEGDA10k MW-DNA, and GelMA80% DoS-DNA hydrogels before and after DNA hairpin-triggered swelling using scanning electron microscopy (SEM) (**Figure 5**a). Before DNA hairpin triggered swelling, the Am-DNA and Am-BIS-DNA hydrogel had pore sizes of several microns, which is consistent with previous reports on Am based DNA hydrogels.[30] Before DNA-directed swelling, the microstructure of all the gels except for the GelMA-DNA gels was similar. The GelMA-DNA gels had smaller visible pores and a denser microstructure than the other gels. Notably, after DNA-directed swelling, the microstructure of all the gels changed dramatically: the gels' pore sizes increased by factors of 5–10. This relative change in porosity for our DNA polymerization motor gel is larger than the change in the porosity of stimuli-responsive DNA hydrogels reported previously,[30a,b] which we attribute to a significantly higher degree of deformation due to a larger extent of swelling in the polymerization motor gels.

We measured the shear storage modulus for the Am-DNA hydrogels as 491.2 ± 17.1 Pa, consistent with the previously reported value.[7] The modulus of the Am-BIS-DNA hydrogels increased significantly to 2255.1 ± 137.3 Pa for Am-BIS-DNA gels with 10 × $10^{-3}$ M BIS [moduli of other Am-BIS(-DNA) gels can be found in Table S2, Supporting Information]. This increase is due to increased cross-linking in bifunctional BIS cross-linked gels.[7,30b,31] We observed that the stiffness of the PEGDA10k MW-DNA hydrogels was significantly higher at 3887.8 ± 493.4 Pa than the modulus of the Am-DNA gels. This modulus could also be tuned by varying the molecular weight of the PEGDA pre-polymer.[18c] This measured modulus was also of a similar order of magnitude as the reported moduli of ethylene glycol diglycidyl ether (EGDE) based DNA hydrogels when corrected for the extent of hydration, which varied in different test conditions.[32] The modulus of the GelMA-DNA gel was 297.0 ± 23.0 Pa, which is significantly lower than the DNA gels mentioned above and similar to the modulus of pure cross-linked GelMA.[19b] Previous studies have established that PEGDA gels have higher modulus as compared to Am and GelMA gels with the same polymer weight fraction due to a combination of chain rigidity, pore size, and extent of cross-linking, and our DNA gels followed the same trend.[19b,33]

We also observed that the moduli for all gels decreased after DNA-directed swelling, which agrees with our SEM observation of the increased sizes of the microstructural pores in the gels after swelling. We hypothesize that such a decrease in modulus can

be caused by increased hydration which reduces the density of the polymer chains.[34] Importantly, our rheology studies convincingly demonstrate that the storage modulus of the DNA polymerization motor gels can be varied by over a factor of 13 between the soft GelMA-DNA gels and the significantly stiffer PEGDA-DNA gels before swelling while remaining stimuli responsive. The ability to tune the modulus of gels is important for applications ranging from tissue engineering to soft robotics.[2c,35]

## 3. Conclusion

In summary, we have demonstrated that DNA polymerization motor gels can be formulated with different copolymers, including Am, Am-BIS, PEGDA, and GelMA of varying composition. We showed how these different gels, while each responsive to DNA signals can have a variety of mechanical and chemical properties. We optimized the recipes for each gel co-polymer synthesis and developed photopatterning protocols, making it possible to assemble these gels at scale in custom shapes. Our photopatterning methods are also compatible with those used to structure previously described shape-change devices, allowing these new gels to be incorporated into architected materials that can change shape through selective deformation in response to DNA stimuli.

From an intellectual perspective, our results validate the idea that the incorporation of DNA hairpin strand displacement and crosslinks into hydrogels might be viewed as a generalized means of creating materials that show high swelling responses to specific biomolecular sequence motifs. As compared to Am-DNA polymerization motor gels, we observed that PEGDA-DNA and Am-BIS-DNA polymerization motor gels are seven to eight times stiffer but still swell appreciably achieving a $\Delta L/L_0$ of at least 0.3, with less stiff gels achieving significantly more response. We note that there is a tradeoff between the extent of swelling and stiffness within Am-BIS-DNA hydrogels. In certain applications such as expansion microscopy, a high degree of swelling is paramount, while in others like soft-robotics modulus can be more important and the higher swelling Am gels tend to rupture more easily.[36] Enhancing the mechanical performance of these materials will extend the application area toward soft robots and machines.[3c,36b,37] We have also shown how DNA-responsive hydrogels with different chemical properties can be readily assembled using the polymerization motor gel concept. GelMA-DNA gels can be utilized for live cell culture during and after DNA hairpin triggered swelling, opening up opportunities for creating cell-laden gels that can respond to specific biomolecular signals while cells grow and divide, providing possibilities in tissue engineering, cell therapy, and for the assembly of bioimplants. We note that our demonstration of GelMA-DNA gels was done to show applicability of the gels in cell culture. Elsewhere, there is significant literature that shows how the modulus of GelMA hydrogels can be varied such as by changing the gelatin methacrylation ratio, and these approaches could be investigated in our gels for broader tissue engineering applications.[19b,38] We also note that a key advantage of our GelMA-DNA hydrogel is that cells can be cultured without functionalizing the polymer with RGD peptides.

We anticipate that these multicomponent DNA-responsive gels might also be used in the assembly of programmed and autonomous untethered smart devices that are fueled or controlled by biomolecular signals. The use of biomolecular sensing and signaling cascades might also allow these structures to be responsive to small molecule signals or specific combinations of biomolecular signals.[13] The ability to program the mechanical properties of these structures can be used to create architected robots that can support weight across a range of size scales or to create compliant domains that deform readily in response to the actuation of softer domains.

## 4. Experimental Section

*DNA Sequences and Preparation*: The DNA crosslinks and hairpins used are listed in Table S1 (Supporting Information). To enable co-polymerization, the DNA crosslink strands were modified with the Acrydite moiety. The DNA sequences used in this study were those reported previously by Cangialosi et al. as System 2 strands.[7] The DNA crosslinks and hairpins were purchased in a lyophilized form from Integrated DNA Technologies (IDT) without additional purification. The DNA crosslinks and hairpins using TAE/12.5 $\times$ $10^{-3}$ M $Mg^{2+}$ buffer (1× TAE buffer was diluted from 50× stock, Life Technologies, #24710-030; magnesium acetate tetrahydrate, Sigma #228648) were suspended and the DNA concentrations were verified using absorbance spectroscopy at 260 nm and were then determined using the extinction constants supplied by IDT. The DNA crosslinks were heated to 90 °C then slowly cooled down to 20 °C using an Eppendorf PCR instrument at a rate of 1 °C $min^{-1}$ at a concentration of 3 $\times$ $10^{-3}$ M per strand. The hairpin strands were diluted to 200 $\times$ $10^{-6}$ M and heated to 95 °C for 15 min and then cooled in ice for 5 min.

*Preparation of the Am-BIS-DNA Pre-Gel Solution*: The concentration of acrylamide as 1.41 M was kept the same in all the Am-BIS-DNA gels, where a 40 wt% acrylamide monomer stock was made using acrylamide from BIO-RAD #161-0100 and MilliQ water. The BIS concentration was varied by adding from a 100 $\times$ $10^{-3}$ M BIS stock solution made from an *N*,*N*′-methylenebis(acrylamide) powder (Sigma-Aldrich, #146072), to make Am-2 $\times$ $10^{-3}$ M BIS-DNA, Am-5 $\times$ $10^{-3}$ M BIS-DNA, and Am-10 $\times$ $10^{-3}$ M BIS-DNA gels, respectively. The concentrations of the other components in the pre-gel solutions were: 1.154 $\times$ $10^{-3}$ M of annealed DNA crosslinks, 3% v/v Omnirad 2100 (formerly known as Irgacure 2100, iGM Resins USA, #55924582) photoinitiator (previously made to a 75% volume fraction of butanol solution), and 2.74 $\times$ $10^{-3}$ M methacryloxyethyl thiocarbamoyl rhodamine B (Polysciences, Inc., #23591) fluorescent dye in TAE/12.5 $\times$ $10^{-3}$ M $Mg^{2+}$ buffer. It was noted that as Omnirad 2100 was a liquid and hence it was easier to mix with the liquid components in the pre-gel formulations. The use of alternate free radical initiators in powder form requires dissolution in solvents such as dimethyl sulfoxide (DMSO) which can induce cell apoptosis and cause denaturation of DNA.[39] Also, Irgacure 2100 has been utilized previously for cross-linking gels for cell culture applications.[40]

The pre-gel solution was well mixed with a pipette and then degassed under vacuum for at least 10 min. The pre-gel solution was made fresh and used immediately for photopatterning.

*Preparation of PEGDA-DNA Pre-Gel Solution*: A 10 wt% of PEGDA hydrogel with 575 (Sigma-Aldrich, #437441), 6k (Sigma-Aldrich, #701963), 10k (Sigma-Aldrich, #729094), and 20k (Sigma-Aldrich, #767549) MW PEGDA monomer was assembled. Other procedures and final concentrations were the same as Am-BIS-DNA gels except that the pre-gel solutions were mixed ultrasonically for 5 min before degassing to allow the Omnirad 2100 to distribute more evenly within the pre-gel solution.

*Preparation of GelMA-DNA Pre-Gel Solution*: The process used to make GelMA-DNA pre-gel solution was the same as the PEGDA-DNA

gel, except the use of 5 wt% of gelatin methacryloyl (300 g Bloom, DoS 80%, Sigma-Aldrich, #900496; 300 g Bloom, DoS 40%, Sigma-Aldrich, #900629) instead of PEGDA monomers, and 2% of Omnirad 2100 instead of 3%. The reduced concentration of GelMA monomer enhanced solubility, and the Omnirad 2100 concentration was reduced to minimize hydrogel cytotoxicity. The buffer content was changed from TAE/12.5 × $10^{-3}$ M $Mg^{2+}$ to sodium phosphate-sodium chloride (SPSC, 1 M NaCl, 50 × $10^{-3}$ M $Na_2HPO_4$; pH 7.5) after the disappearance of the DNA hydrogel in $Mg^{2+}$ buffer was found. The pre-gel solution was heated to 60 °C before sonication to prevent it from gelling.

*Lithography Preparation and Photopatterning*: Photolithography chambers were assembled as reported previously.[7,18c] Briefly, the chamber consisted of a glass substrate (bottom) and a Cr mask (top) to selectively expose UV light through the transparent part of the mask and initiate a free radical chain polymerization reaction in the pre-gel solution. The Cr mask was prepared by spin coating SC1827 (Microchem, Microposit S1800 Series) at 3000 rpm for 5 min and then baked on a 110 °C hot plate for 1 min, followed by curing with 180 mJ $cm^{-2}$ dose of 365 nm UV light through AutoCAD-designed plastic masks (printed by Fineline Imaging). The glass slide was then developed using a 1:5 w/w solution of Microposit 351 Developer (Shipley) and water. After drying in air, a 150 nm thick layer of Cr was deposited onto the glass slides using thermal evaporation. The slides were washed with acetone and isopropyl alcohol to remove the unexposed photoresist. To pattern square DNA hydrogel single-layer films, 1 x 1 mm square masks were prepared as described above. The thickness of the layers could be tuned using different thicknesses of spacers (160 µm in this paper). The pre-gel solution was injected into the chamber and then exposed to a 365 nm UV light source (Neutronix Quintel aligner). The energy doses for Am-DNA, Am-BIS-DNA, PEGDA-DNA, GelMA-DNA hydrogels were 160, 160, 600, 200 mJ $cm^{-2}$, respectively. After UV exposure, the chamber was gently disassembled, and a TAE/$Mg^{2+}$ buffer was used to wash out the extra pre-gel solution and hydrate the gel. The hydrogel was then stored in the TAE/$Mg^{2+}$ buffer at 4 °C to achieve complete hydration before DNA-directed swelling experiments; the portion of intrinsic swelling with the buffer was not included in the swelling kinetics calculations.

*Swelling Experiments*: The extent of swelling of the hydrogel was recorded using time-lapse fluorescence imaging with a gel imager (Syngene EF2 G:Box) equipped with a blue light transilluminator (Clare Chemical, max emission at ≈450 nm) and a UV 032 filter (Syngene, bandpass 572–630 nm). The hydrogel samples were transferred to wells within a 48 or 96 well plate to isolate the gels from each other while swelling. In this paper, the concentration of DNA hairpin monomers was diluted to 20 × $10^{-6}$ M for each strand, with 98% of polymerizing monomers and 2% terminator monomers using a TAE/12.5 × $10^{-3}$ M $Mg^{2+}$ buffer or sodium phosphate-sodium chloride (SPSC, 1 M NaCl, 50 × $10^{-3}$ M $Na_2HPO_4$; pH 7.5) buffer (only for GelMA-DNA gels) with 0.001% TWEEN20 (Sigma, #051M01811V). Images over 30-min time intervals were taken for at least 3 days. The side lengths of the DNA hydrogels using MATLAB or manually using ImageJ were quantified when the gels were not easily identifiable because they had moved during swelling or had low fluorescence intensity. It was chosen to use $\Delta L/L_0$ because length is the commonly used parameter to display hydrogel size change in prior literature, including in our own work, and it can be easily and intuitively scaled to both $\Delta$volume/volume$_0$ and $\Delta$area/area$_0$.[7,41] At least three samples were used to generate the mean value and standard deviation for each swelling kinetics curve. The solid curves represented the changes in length with respect to the original side length, and the dotted curves around each solid curve represented the standard deviation.

*Cell Culture*: HeLa/GFP reporter cells were purchased from Cell Biolabs Inc. (#AKR-213). After defrosting, the cells were cultured using a DMEM medium (Corning Cellgro, 10-013-CV) containing 10% FBS (ThermoFisher, 26140079) and 1% Penicillin-Streptomycin (ThermoFisher, 15140122). 5–10 mL of cells were cultured in 25 $cm^2$ culture flasks (Falcon, 353109) at 37 °C with 5% $CO_2$. HeLa cells were released from the flask surface using 1 mL 0.05% Trypsin-EDTA (ThermoFisher, 25300054) and split every 2 days. When culturing cells on the gels, 10 × $10^{-6}$ M of DNA hairpin solution was prepared with media as described above. This lower concentration of hairpins was used to minimize apoptosis that can occur with high concentrations of DNA hairpins. Nearly $10^5$ cells were passed onto one well of a 96 well plate with 200 µL of media. After seeding, the hydrogel was transferred to a clean well with culturing media containing DNA hairpins for better imaging. Fluorescence images were taken using a Nikon AZ100 multi-zoom epifluorescence microscope with a Nikon DS-Fi1 camera. Fluorescence images of HeLa/GFP cells were captured using a Nikon B-2E/C filter cube (excitation 465–495 nm, bandpass emission filter 515–555 nm), whereas images for GelMA-DNA gels were captured using a Nikon G-2E/C filter cube (excitation filter 528–553 nm, bandpass emission filter 590–650 nm). There was minimal movement of the gels during the imaging period, and the gels were washed with media before every image.

*Sample Preparation for Shear Modulus Measurements*: A 1 mm thick negative PDMS mold with cylindrical holes was made using a 6 mm hole punch and then stuck to a clean glass slide. The hydrogel pre-gel solution was made as described above only without rhodamine B and was injected into the holes and polymerized using 365 nm UV light with an intensity of 4.0 mW $cm^{-2}$ for 20 min to ensure complete curing. Then the samples were soaked in a TAE/$Mg^{2+}$ buffer overnight to hydrate the gels fully. Three samples of each type of hydrogel were tested for calculating the mean value and standard deviation. The shear modulus of the hydrogel samples was measured using a TA instrument rheometer (AR1500EX) with an 8 mm plate. A solvent trap attachment was used to prevent the hydrogel from dehydrating during the measurement. Sandpaper was glued to both surfaces of the plate to prevent slippage between the gel and the plate. Time sweeps were used with a 1% strain at a 2 Hz frequency. After testing the as-prepared gel, the gel was soaked in 20 × $10^{-6}$ M of DNA hairpin solution and used again for testing after 1 and 2 days.

*SEM Imaging*: Hydrogel samples were prepared as described in the previous section (Sample Preparation for Shear Modulus Measurements). The gels were allowed to hydrate in TAE/$Mg^{2+}$ buffer overnight or were swollen in TAE/$Mg^{2+}$ buffer containing 20 × $10^{-6}$ M of corresponding DNA hairpin solution with 2% terminator hairpins for 2 days. The gel samples were then frozen in a –80 °C refrigerator for 24 h and lyophilized for 2 days to fully remove the water. The samples were then preserved at 4 °C under nitrogen gas until imaging. SEM images were taken using a JEOL SEM (JSM IT100), and the gels were cut with a razor blade, sputter-coated with gold to enhance contrast and imaged on the cross-section.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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## Conflict of Interest

We have a patent application filed.

## Author Contributions

R.S., R.S., and D.H.G. conceived this study. R.S. performed synthesis and experiments. R.S. and J.F. processed the data. W.X. and Q.H. helped characterizing the gels using scanning electron microscopy. S.J. and G.P. helped with cell culturing. R.S., R.S., and D.H.G. wrote the paper with input and edits from all authors. Some of the authors are listed as inventors on a patent pending on the DNA polymerization motor gels.

## Keywords

DNA nanotechnology, hydrogels, shape change, soft robotics, tissue engineering

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