Multi-domain Automated Patterning of DNA-Functionalized Hydrogels

Moshe Rubanov\textsuperscript{a}, Joshua Cole\textsuperscript{a*}, Heon-Joon Lee\textsuperscript{a*}, Zachary Chen\textsuperscript{a}, Elia Gonzalez\textsuperscript{a} and Rebecca Schulman\textsuperscript{a,b,c}\textsuperscript{*}

\textsuperscript{a}Department of Chemical and Biomolecular Engineering, Johns Hopkins University, 3400 N. Charles Street, Baltimore, Maryland 21218, United States

\textsuperscript{b}Department of Computer Science, Johns Hopkins University, 3400 N. Charles Street, Baltimore, Maryland 21218, United States

\textsuperscript{c}Department of Chemistry, Johns Hopkins University, 3400 N. Charles Street, Baltimore, Maryland 21218, United States

\textsuperscript{*}Corresponding Author: rschulm3@jhu.edu

Abstract

DNA-functionalized hydrogels are capable of sensing oligonucleotides, proteins, and small molecules, and specific DNA sequences sensed in the hydrogels’ environment can induce changes in these hydrogels’ shape and fluorescence. Fabricating DNA-functionalized hydrogel architectures with multiple domains could make it possible to sense multiple molecules and undergo more complicated macroscopic changes, such as changing fluorescence or changing the shapes of regions of the hydrogel architecture. However, automatically fabricating multi-domain DNA-functionalized hydrogel architectures, which could enable the construction of hydrogel architectures with tens to hundreds of different domains. We describe a platform for fabricating multi-domain DNA-functionalized hydrogels automatically at the micron scale, where reaction and diffusion processes can be coupled to program material behavior. Using this platform, the hydrogels’ material properties, such as shape and fluorescence, can be programmed, and the fabricated hydrogels can sense their environment. DNA-functionalized hydrogel architectures with domain sizes as small as 10 microns and with up to 4 different types of domains can be automatically fabricated using ink volumes as low as 50 \( \mu \)L. We also demonstrate that hydrogels fabricated using this platform exhibit responses similar to those of DNA-functionalized gels fabricated using other methods by demonstrating that DNA sequences can hybridize within them and that they can undergo DNA sequence-induced shape change.

Introduction

DNA is a versatile tool for performing chemical information processing and storage, and for executing temporal and spatial chemical programs \((1,2)\). DNA can be used to write molecular programs, \textit{i.e.}, sets of reaction networks that together process chemical information, such as the concentrations or types of chemicals present in a solution. Molecular programs can have multiple inputs and outputs \((3)\). Molecular programs can also be localized, \textit{i.e.}, specific molecules can be anchored in place, and interact with diffusing molecules to produce spatiotemporal molecular programs. Spatiotemporal molecular programs can sequentially release DNA at prescribed times and locations, or generate stable chemical gradients within a microfluidic chamber \((4–6)\). The DNA molecules that specify a spatiotemporal program can be conjugated to substrates such as hydrogels, surfaces, colloids, cell surfaces, or proteinosomes \((3,7–11)\). In such systems, spatiotemporal molecular programs can swell materials, cause them to assemble
into 2 and 3-dimensional shapes or alter their fluorescence or porosity (3,12–14). For example, the DNA attached to DNA-functionalized colloids can direct how these colloids self-assemble into specific nanostructures (11,15) and DNA-embedded proteinosomes can communicate with their neighbors (9). Genes can be expressed locally on DNA-functionalized surfaces (16) and DNA-embedded hydrogels can transform input patterns of light into spatial patterns of DNA species (17).

DNA within DNA-functionalized hydrogels can react with free DNA, induce programmable shape change, and can be transcribed and translated for cell-free protein synthesis (14,18–20). DNA-functionalized hydrogels can likewise act as sensors and transducers to enable the translation of sensory information in the environment to a change in the hydrogel (21). For example, a DNA-functionalized hydrogel-based sensor can simultaneously detect and remove mercury from water (22) and sensors for DNA-based hydrogels exist for inputs such as miRNA, pH, ATP, temperature, aptamers, and many more (23). Multidomain hydrogel architectures can likewise be used to create shape-changing materials with multi-stimulus control, or as sensors capable of integrating multiple stimuli (21,24).

Here we sought to develop a platform for automatically fabricating multi-domain DNA-functionalized hydrogels with low reagent volumes (100 µL) and high resolutions (down to 10 µm). This platform would enable the fabrication of hydrogels that could compartmentalize DNA within a larger architecture, leading to greater functionality for these hydrogel architectures as shape-changing materials or sensors.

Many different methods for fabricating multi-domain hydrogels have been developed (25). Extrusion-based hydrogel printing has been used to print 3D multi-domain hydrogels down to 100s of microns (26). For higher resolutions (10s of microns), light-based methods for fabricating hydrogels are generally used. One light-based method for fabricating hydrogels involves digital light processing (DLP), where a digital micromirror device is used to direct light to photopolymerize hydrogels at different locations. Hydrogels fabricated with DLP can have resolutions down to 7 microns (27). However, scaling the number of inks within DLP systems remains challenging, as the hydrogels are generally fabricated from within an open vat, and exchanges of inks within a vat are difficult. There exist a few examples in the literature where vat exchange enables the automated patterning of multi-domain 3D hydrogels (28,29). However, to scale this method towards 10s or even 100s of inks, having a vat for each ink would be costly and impractical. Another example is to exchange inks within a closed vat using a flow controller (30). This method, however, requires milliliters of each ink. We aim to adapt high-resolution DLP-based photopatterning towards a hydrogel fabrication platform that has a scalable number of inks while keeping ink volumes down to 100s of microliters.

Here we develop a platform termed Multi-domain, Automated Photopatterning of DNA-functionalized Hydrogels (MAPDGH) which enables the automatic fabrication of multi-domain DNA-functionalized hydrogels within a microfluidic chamber using low ink volumes (down to 50 µL) with an integrated Python script. We characterize the reproducibility of fabricating DNA-functionalized hydrogels between each patterning round (injecting new ink) by measuring average fluorescence and hydrogel length. We then demonstrate the ability to fabricate a four-color pixel-art image by photopatterning four different DNA-functionalized hydrogels in specified locations (i.e., pixels) extracted from the image. To test whether DNA anchored within fabricated hydrogels can react with free DNA, we fabricate another four-domain hydrogel architecture, where two of the four DNA-functionalized hydrogels, anchored with fluorescent DNA, can hybridize with a quencher-modified DNA strand that is flowed in after patterning.
Finally, we program domain-specific hydrogel swelling in fabricated DNA-functionalized hydrogels by adapting recipes for DNA-crosslinked hydrogels from Shi et al. (18) that can undergo addressable swelling based on the DNA hairpins added to the solution surrounding the hydrogel. In summary, we demonstrate a hydrogel fabrication platform that can fabricate multi-domain DNA-functionalized hydrogel architectures capable of addressable hybridization and DNA-induced swelling.

**Materials and Methods**

The protocol described in this peer-reviewed article is published on protocols.io, https://dx.doi.org/10.17504/protocols.io.j8nlkw2yw5r/v1 and is included for printing as supporting information file 1 with this article.

**Results**

Here we present a new method for automated patterning of DNA-functionalized hydrogels, which we term Multi-domain Automated Photopatterning of DNA-functionalized Hydrogels (MAPDH). MAPDH is a photopatterning method which fabricates architected DNA-crosslinked hydrogels within a microfluidic chamber (Fig 1A) using an integrated Python script (Fig 1B, SI Sections 1,2) (31). A Python script interfaces with all MAPDH hardware – it is used to control a digital micromirror device (DMD), a UV LED, a microscope XY stage, and a custom-built pneumatic flow controller. A Python script can specify which vial to flow ink from (FLOW()), where to pattern (MOVE(DOMAIN[])), as well as the shape to pattern with (PATTERN(DOMAIN[])) (SI Sections 1,2). The patterning method is sequential – we first flow in a particular ink, move the chamber to a specified location, upload a mask, then expose the chamber to UV light, which polymerizes the ink to form a hydrogel domain or domains. Each ink is a solution that consists of a monomer, a photoinitiator, and specific acrydite-labeled DNA strands. The DNA strands can also include modifications, such as a fluorophore that allows for visualization. After hydrogels with specified shapes and locations are patterned using a particular ink, an automated wash step is applied which removes the ink. Another flow step can then be started to flow in a new ink from a separate vial (Fig 1C). This process can be repeated and can use up to 4 different inks in 4 different vials. A schematic of this process is described using the syntax in Fig 1B. A schematic of how this syntax describes the series of ink flow, patterning, and washing steps during an MAPDH is shown in Fig 1C.

**Figure 1: Multidomain, Automated Photopatterning of DNA-functionalized Hydrogels (MAPDH).** A Generalized workflow for fabricating DNA-functionalized hydrogels. The hydrogel location, size, shape, and composition are specified, after which an automated script is run, and the output is a chamber with photopatterned hydrogels of different sizes, shapes, and compositions. B Pseudocode for MAPDH in Python. The algorithm takes as inputs the vials that will be flowed through the patterning chamber. The algorithm also takes as input the hydrogel location, as well as the mask (and thus the shape) of each hydrogel. The algorithm
incorporates a wash step between each round of patterning. C Workflow for MAPDH. After fabrication, inks are flowed and patterned sequentially, with an automated wash step between each patterning step. These domains can be patterned with interpenetrating borders, or as standalone hydrogels. D MAPDH Setup. Pressurized air (1) is controlled by a pressure regulator (2) to 10 PSI. The air from the regulator is split to 5 different solenoid electronic valves (3), which are initially closed. When open, the air from one of the electronic valves is flown through a pressure gauge (4) and injected into an air-tight vial (5) above the ink. Another tube, located at the bottom of the conical vial, is filled with ink. When the electronic valve is turned on, the pressurized air pushes on the air inside the ink vial, which forces ink up the second tube (6) (SI Section 3). The second tube is fed directly into a microfluidic device (7), and after patterning the ink exits through an outlet into a waste receptacle (8). To photopattern hydrogels in place, we shine UV light (11) towards a digital micromirror device (10), where the light is reflected at specified locations where the mirrors are activated, which enables the shaping of light, and focused through an objective (9) onto the microfluidic patterning chamber. The microfluidic chamber has 5 inputs (12), followed by 5 microfluidic resistors (13), which prevent backflow (SI Section 4). The patterning chamber (14) consists of 2 rows for photopatterning, followed by an outlet (15).

DNA-functionalized hydrogels can be fabricated using a manual photopatterning technique that required manual ink injections, manual UV exposures, manual location movement and manual mask uploading (17,32–34). When a number of patterning steps are required, this method can be time-consuming. It can also require the use of milliliter concentrations of inks, which can be prohibitively expensive when inks contain, for example, high concentrations of modified DNA. We created MAPDH to fabricate DNA-functionalized hydrogels with less tedium and much lower volumes of inks.

MAPDH uses an electronically controlled, pneumatic, low-dead volume microfluidic flow controller to direct automatic influx of inks and washing fluid into a microfluidic chamber and controls how regions of the chamber are exposed to light using a digital micromirror array (Fig 1D) (SI Section 3) (35). Pressurized air provides the driving force (Fig 1D1) directly fed into a pressure regulator (Fig 1D2) that controls the pressure to 5 different electronically controlled pneumatic solenoid valves (Fig 1D3). Pressure to each vial is precisely and modularly controlled using fine pressure gauges (Fig 1D4). The fine pressure gauges are then connected directly to ink vials, and the pressure applied to the ink vials controls the corresponding flow rate of ink from that vial. The inks are housed in 1 mL air-tight conical vials with two drilled holes in the lid. Pressurized air from the pneumatic flow controller enters in an air input tube through one hole into the air above the ink inside the vial. The second hole houses an ink output tube where ink solution is output into the chamber when air pressure is applied to the vial through the air input tube (Fig 1D5). By opening the solenoid valve, the ink is driven through the second tube and out of the vial. To minimize the dead volume for MAPDH of each ink the ink output tube starts at the very bottom of the conical vial, so that patterning occurs reliably when a vial contains as little as 100 µL of ink (SI Section 3). The ink output tube (Fig 1D6) feeds directly into a microfluidic chamber (Fig 1D7). The microfluidic chamber consists of annealed PDMS onto glass and has 5 input ports for 4 inks and 1 wash solution (Fig 1D12, SI Sections 4,5). After photopatterning, wash solution flushes the cell, removing the ink. This waste exits the chamber through a waste tube (Fig 1D8) (Fig 1D9). We expose the microfluidic chamber to UV light that is focused through an objective (Fig 1D9) to achieve photopatterning resolution on the order of 10s of microns. The UV light (Fig 1D11) is shaped using a digital mask uploaded to a Digital Micromirror Device (DMD) (Fig 1D10) and the light is projected onto the chamber to precisely control hydrogel photopolymerization and shape. Microfluidic resistors prevent backflow from the microfluidic chamber into the ink output tubes (Fig 1D13). The region of the chamber where patterning occurs consists of two chambers separated by a 100 µm thick PDMS wall (Fig 1D14).

We initially characterized the shapes of hydrogels and the concentrations of DNA within PEGDA-co-DNA hydrogels fabricated using MAPDH. We setup a MAPDH protocol, which consists of multistep fabrication of DNA-functionalized hydrogels using an automated workflow and multiple vials (SI Section
We fabricated hydrogels containing fluorescent DNA and then measured the shapes of the hydrogels in fluorescence micrographs and the fluorescence intensities within the shapes. Initially, we fabricated 5 hydrogels with the same ink using MAPDH, with a target length of 50 µm. The resultant lengths for these 5 hydrogels are 51.5 µm ± 0.6 µm (mean ± S.D.), which was within our goal for accurately fabricating hydrogels within a resolution of 10s of microns (SI Section 7).

We next sought to measure the variation in hydrogel shape and DNA concentration between patterning rounds, where a patterning round consists of 1) ink flow, 2) printing, and 3) washing. The printing step (#2) consists of one or more cycles of a) moving the chamber to a location, b) flowing the ink used in #1 for a few seconds, and c) photopatterning. The purpose of b) was that we found that flowing the ink needed for patterning for a few seconds just before photopatterning led to low variance in the patterned sizes of hydrogels (SI Section 6).

To measure the amount of variation in fabricated hydrogel size, we set up a MAPDH protocol with 12 patterning rounds, in which a column of 5 rectangular hydrogel posts were patterned in each round. The 12 rounds used were VIALS = [1,2,3,4,1,2,3,4,1,2,3,4], respectively (Fig 2A-G) (SI Section 6). After each column of 5 hydrogels was patterned, the chamber was washed using the buffer wash solution (1x TAE Mg²⁺). There was no significant difference between the average lengths or fluorescence intensities of the hydrogels patterned in each round (Fig 2H,I, SI Section 7). Thus, fabricating hydrogels with different vials and/or inks using MAPDH can be done interchangeably without affecting hydrogel properties such as length or fluorescence.

We then explored the ability to fabricate hydrogels smaller than 50 µm in length: we were able to fabricate hydrogels using a 20 and 10 µm length square masks with average lengths of 27.1 ± 0.7 µm and 13.69 ± 0.7 µm (mean ± S.D.), respectively, using a modified version of MAPDH (SI Sections 8,9). Finally, we asked whether we could pattern multi-domain hydrogel systems containing different types of hydrogels: we added 4 different types of inks with 4 different acrydite- and fluorophore-modified DNA to 4 different vials; all the patterned hydrogels had the specified fluorescence intensities in the 3 different fluorescent channels (Fig 2J, SI Sections 10,11).

Figure 2: MAPDH Characterization. A Pseudocode describing automated workflow. 4 inks were patterned 3 times for a total of 12 rounds. Each round consists of patterning 5 rectangular hydrogels in a column before moving to the next column. B-F: Micrographs of the patterning progression. B patterning the first hydrogel during the first round. C patterning the second hydrogel during the first round. D completing the first patterning round. E completing the second patterning round. Round 1 used vial 1 and round 2 used vial 2, which both contained the same ink. F completing 4 patterning rounds. Vials 1, 2, 3 and 4, respectively were used during the 4 rounds. G completing all 12 patterning rounds using the program in A. H Average length for each hydrogel within a round. Error bars are standard deviation in length per hydrogel within a round. I Average Fluorescence.
for each hydrogel within a round. Error bars are standard deviation in length per hydrogel within a round. 

Combined micrograph of 4 different hydrogel domains using 4 ink compositions in separate vials. The inks each contained 100 nM of acrydite-modified, fluorophore-modified DNA, except for the white domain, which contained 100 nM of all 3 DNA strands. Ink compositions are shown in SI Sections 6,10. B-G were run using a MAPDH script in SI Section 6. J was run using a separate MAPDH script in SI Section 10. Scale bars are 100µm.

To test the scalability of the MAPDH system, we next created a multi-domain hydrogel “image” consisting of individually patterned rectangular hydrogel “pixels”. We chose a 15x15 pixel art image of a watermelon that contained four unique colors (Fig 3A). To develop a MAPDH protocol for this hydrogel architecture, we wrote an algorithm that constructed four 15x15 binary matrices specifying the location to pattern each color in the watermelon image, corresponding to four hydrogel domains (Fig 3B, SI Section 12). The locations extracted from the image were then converted into locations for patterning during MAPDH which were then automatically converted into a MAPDH protocol to construct the watermelon grid (SI Section 13). We observed no significant misalignment between the patterning of each individual gel pixel – the hydrogels were precisely patterned at their specified locations, with no overlap between neighboring hydrogel pixels. Since we had three orthogonal wavelengths to image the various fluorophores in, the fourth ink (represented as pink in Fig 3A,B) was a combination of all three fluorophores. We false-colored the composite micrograph based on the colors in the original pixel-art image. In the final hydrogel architecture, dark green corresponded to 500nM of 5Acry_3Cy3_polyT10, light green corresponded to 500 nM of 5Acry_3ATTO488_polyT10, red corresponded to 500 nM of 5Acry_3TYE665_polyT10, and pink corresponded to 500 nM of all three acrydite- and fluorophore-modified strands (SI Sections 14,15).

Figure 3: Fabricating a pixel-art based 4-domain DNA-functionalized hydrogel. A We chose a 15x15 pixel art image consisting of four colors. B Using an automated script, the four colors are distributed into four 15x15 binary matrices storing the locations for patterning of each domain. C The four binary matrices are then fed into a MAPDH protocol designed to take as input a set of locations for each ink to pattern. In this case, the fourth ink contained a combination of all three fluorophores, resulting in hydrogels patterned in that domain visible in all three imaging channels. A colormap was applied to each fluorescent micrograph to match the color on the original pixel art image. D The fluorescent micrographs are then combined for a composite micrograph shown in D, with the pink domain (the fourth ink patterned) colored based on fluorescence intensities from all three fluorescent micrographs. Scale bars are 100µm.

Next, we asked whether hydrogels fabricated through a MAPDH protocol could, by controlling where DNA is conjugated within hydrogels in an architecture, control where hybridization reactions occur. This would make it possible to control where DNA circuits operate or where other DNA-mediated
downstream processes take place (4,12,14,36). We fabricated a hydrogel architecture with 4 gels, each a different domain. One of the gels in the architecture, a circle, contained a fluorescently labeled DNA molecule 5Acry_3Cy3_R1. A second gel, a square, contained 5Acry_3Cy3_R1 and two other DNA species labelled with Atto488 and TYE665. The two other gels, a triangle and a plus sign, had other DNA species labeled with Atto488 and TYE665 (Fig 4A). The fluorescence produced by 5Acry_3Cy3_R1 could be quenched through hybridization by a complementary sequence bearing a fluorescence quencher, 5Q_R1’ (SI Section 16).

After this hydrogel architecture was fabricated, a solution containing 5Q_R1’ was flowed into the microfluidic chamber. We then observed that the hydrogel that contained only 5Acry_3Cy3_R1 (the green circle) decreased in fluorescence intensity and the fluorescence intensity of the hydrogel containing all three fluorophore-modified DNA strands decreased in the channel corresponding to the 5Acry_3Cy3_R1 label (Cy3), but not in the other two observed fluorescence channels. The fluorescence intensities of the other two hydrogels did not change after 5Q_R1’ was added to the microfluidic chamber (Fig 4B, SI Section 17). Thus, DNA in solution can hybridize with DNA conjugated to MAPDH-fabricated hydrogels, enabling addressable control of DNA hybridization within hydrogel architectures.

Figure 4: Addressable hybridization reactions in MAPDH-fabricated hydrogel architectures. A Schematic illustrations of each domain in the hydrogel architecture showing the acrydite- and fluorophore-modified DNA each domain contains. The arrangement of the domains in the illustration is also their designed arrangement in the fabricated hydrogel architecture. The ink for the circular hydrogel post contained 500nM of 5Acry_3Cy3_R1 (labeled as a green ink), the ink for the triangular hydrogel post contained 500 nM of 5Acry_3ATTO488_polyT10 (labeled as a blue ink), the ink for the plus-shaped hydrogel post contained 500 nM of 5Acry_3TYE665_polyT10 (labeled as a red ink), and the ink for the square hydrogel post contained all 3 of the listed DNA strands, each at 500 nM. The fabricated circular and square posts thus contained DNA that could hybridize to quencher-modified DNA strand (5Q_R1’), the complement of 5Acry_3Cy3_R1, when solution containing 5Q_R1’ is added to the chamber (sequences in SI Section 34). B Composite multicolor fluorescence micrographs before and after addition of solution containing 500 nM 5Q_R1’. The square post appears white before hybridization because it has high green, blue and red fluorescence intensity, but magenta after adding the solution containing 5Q_R1’, because it has high blue and red fluorescence intensity but less green fluorescence intensity. Scale bars are 100 µm.

We next sought to develop a method of fabricating free architected hydrogels by fabricating hydrogels in a microfluidic device, lifting them off the surface, removing them from the device and collecting them. We call this method MAPDH-LC, or Multi-Domain Automated Photopatterning of DNA-
Functionalized Hydrogels with Lift-off and Collection. We began by developing MAPDH-LC for fabricating single-domain hydrogels and then detaching them from the device surface.

This method uses a sacrificial layer that after patterning is controllably dissolved to lift-off and remove hydrogels from a modified microfluidic chamber, termed the lift-off flow chamber (Fig 5A). Prepolymer solution for the sacrificial layer is poured into a PDMS 'boat' for thermal curing (SI Sections 18,19). After thermal curing, the PDMS boat with the sacrificial layer is sandwiched with a second PDMS structure containing the patterning chamber to form a lift-off flow chamber (Fig 5B, SI Section 20). We chose poly(acrylic acid) (PAA) for the sacrificial layer due to PAA’s ability to selectively dissolve in NaCl (37).

To test this method, we first fabricated 25 simple rectangular PEG hydrogels containing 5Acry_3Cy3_polyT10, so that the resulting hydrogels would be fluorescent (Fig 5C). After patterning, a 1 M NaCl solution was then automatically flowed into the chamber to detach the gels from the sacrificial layer within the chamber. To extract the detached hydrogels, we flowed the same buffer solution used for the ink solution, 1x Tris base, acetic acid, EDTA, 12.5 mM Mg$^{2+}$ (1x TAEM) to remove the hydrogels from the device via convection (SI Section 21).

After the free hydrogels were extracted from the device, we developed a protocol for collecting the hydrogels that involved concentrating the hydrogels in a small volume of buffer before transfer into a 96-well plate. The hydrogels that had been removed from the device were washed into a microcentrifuge tube with 1mL of 1x TAEM buffer solution. The microcentrifuge tube containing the hydrogels was then centrifuged for a few seconds so that the hydrogels were concentrated at the bottom of the tube. After centrifugation, 20 µL of the bottom of the solution was then pipetted into a 96-well plate for observation and further analysis (SI Section 21).

After collection, we observed 13 free hydrogels within the 96 well plate. Of those 13 hydrogels, we measured the lengths of one side of 7 hydrogels that lay flat on the surface of the 96-well plate (SI Section 22). We compared the lengths of the 7 hydrogels measured after collection to the side lengths of 7 randomly selected gels within the chamber just after patterning (i.e., before liftoff) (Fig 5D). The side length (mean ± s.d.) of the hydrogels after lift-off and collection was 189±14 µm, and the mean side length of hydrogels just after patterning was 195±9 µm. These results suggest that this process could be used to fabricate free gels of a specified size.

We next asked whether we could extend MAPDH-LC to fabricate free multi-domain hydrogels. We considered a target three-domain hydrogel composed of three adjacent 200x200 micron domains (Fig 5E, SI Section 23). We organized the domains so that adjacent patterned areas each overlapped by 10 microns.

We first asked whether we could successfully lift three-domain hydrogels off the device surface after performing multiple rounds of patterning using MAPDH. We attempted to fabricate 25 three-domain hydrogels in three patterning rounds where we patterned each of the three types of domains. We observed that 12 of the 25 three-domain hydrogels were washed away during one of the three patterning rounds. The 13 hydrogels that remained after the third patterning round were removed from the device surface during the step of washing with 1 M NaCl (SI Section 23).
We next asked whether we could recover three-domain hydrogels (rather than individual separate domains) using this process by patterning the domains of the hydrogels as overlapping regions. We wanted to see whether the 10-micron overlap was sufficient to keep the hydrogel domains together during the lift-off and collection process. Of the 13 three-domain hydrogels that remained adhered to the PAA surface before lift-off, we successfully collected two into a 96-well plate (SI Section 24). We observed that for both hydrogels collected, the three domains remained attached to each other after collection, suggesting that the interfaces created during patterning were mechanically strong enough to prevent breaking during lift-off and collection (Fig 5F).

DNA-crosslinked PEG hydrogels can swell in response to specific DNA sequences (14). We asked if we could use MAPDH-LC to fabricate DNA crosslinked hydrogels and whether these gels would swell in response to DNA sequences.

To fabricate DNA-crosslinked hydrogels using MAPDH-LC, we adapted an ink formulation used for photopatterning DNA-crosslinked PEGDA-10K hydrogels from Shi et al. (18) (Fig 6A). We changed the photoinitiator and exposure time, while keeping the monomer (PEGDA-10k) and DNA sequences and concentrations identical. We replaced the photoinitiator originally used in that work with Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) and the exposure time was reduced to 1 second (SI Section 25).
Using this ink, we were able to fabricate well-formed hydrogels and lift them off and collect them. Of the 30 gels that were photopatterned, 18 gels were collected. Of these 18 gels, 11 lay flat on the surface (without bending or wrinkling) in the 96-well plate where the gels were collected, meaning that if they swelled, the extent of swelling could be measured easily by measuring the changes in their lengths (SI Figure 19). We next swelled the gels and used the 11 that laid flat to measure swelling extent over time (SI Section 26).

To swell the gels, we added two DNA swelling signals (System 1 hairpins, S1_H1 and S1H2) at 20 μM each to the solution surrounding the free hydrogels, initiating the DNA hybridization chain reaction that drives hydrogel swelling. We measured the amount of swelling of 11 one-domain hydrogels over 60 hours in terms of the lengths of the hydrogels at a given time divided by their original lengths, (ΔL/L₀) (Fig 6B). The ΔL/L₀ after 60 hours was 0.51 ± 0.010 (mean ± s.d.) (Fig 6B), which is slightly larger than the ΔL/L₀ of approximately 0.4 after 60 hours reported in Shi et al. (18), (SI Sections 27,28). Thus, MAPDH-LC can be used to fabricate and collect single-domain DNA-crosslinked hydrogels that then subsequently swell in response to specific DNA sequences.

We next attempted to use MAPDH-LC to fabricate and collect 3-domain DNA-crosslinked hydrogels where different domains within this structure swell in response to different DNA sequences (Fig 6C). Two of the domains had two different DNA-crosslinks within them (red and blue in Fig 6C), while the third domain did not contain any DNA crosslinks. Thus, the red domain should swell in response to one type of sequence (termed the red swelling signal), the blue to another (termed the blue swelling signal) and the green domain should not swell in response to any DNA stimuli. We used MAPDH-LC (Fig 5E) to pattern 50 three-domain hydrogels. We were able to successfully collect just one free 3-domain hydrogel (SI Section 29).

To test whether the patterned domains were selectively responsive, we actuated the red and blue domains sequentially by adding in red and then blue swelling signals. We observed swelling of the red domain for the first 10 hours after addition of the red DNA swelling signal (system 1 hairpins) (Fig 6D). After about 24 hours, we added the blue-domain DNA signal (system 2 hairpins) to initiate swelling of the blue-domain hydrogel (Fig 6D, SI Section 30). We observed that the swelling of the red and blue domains were constricted near the edge shared with the green domain. To quantify the amount of swelling, we chose to measure the changes in lengths of the edge opposite the green domain to measure ΔL/L₀. After 60 hours, this ΔL/L₀ was 0.4 for the red domain and 0.6 for the blue domain (Fig 6E) (SI Section 31), consistent with the swelling observed for single-domain hydrogels. The green domain did not appear to swell, but it bowed outward over time near its edges contacting the swelling domains (Fig 6D), likely due to the stress applied by the swelling of the blue and red hydrogel domains. We also measured the differences in extent of swelling between the edges of the red domain closest and farthest from the green domain, which was 0.217. This was approximately 50% of the final ΔL/L₀ observed, suggesting some control over swelling within a hydrogel domain by constraining regions of a hydrogel domain with non-swellable domains.

These results indicate how control and restriction of swelling is possible by patterning DNA-crosslinked domains that swell in response to different signals using MAPDH-LC. Because MAPDH-LC can be used to pattern complex multi-domain architectures, MAPDH-LC could be used to readily fabricate hydrogels that programmatically morph into different 2D- or 3D-shapes.
Here we demonstrated an integrated method for multi-domain DNA-functionalized hydrogel fabrication, MAPDH. We could reproducibly pattern hydrogel domains in multiple rounds and fabricate hydrogels with shapes and compositions with sizes ranging from 10s to 100s of microns. We showed the ability to fabricate 4-domain DNA-functionalized hydrogels to recreate a pixel art image with precise alignment between hydrogel pixels. DNA oligos can diffuse into the patterned hydrogels, where they can engage in local hybridization reactions with DNA functionalized to the patterned hydrogels. Finally, we developed a method for selectively removing the hydrogels from the substrates after patterning using a dissolvable sacrificial layer. We demonstrated the ability to pattern and remove a three-domain interconnected DNA-functionalized hydrogel architecture from the patterning chamber and selectively swell hydrogel domains using a hybridization chain reaction in a separate container.
MAPDH is currently capable of printing 4 unique DNA-functionalized hydrogel domains, with a 5th vial required for washing the patterning chamber between patterning steps. Due to the modular nature of the flow controller, we envision future versions of MAPDH having much greater numbers of inks. To expand the number of inks, the pneumatic flow controller can be expanded, and the microfluidic chamber can be redesigned to accommodate a greater number of inlets. A future version of MAPDH could have 10s or even 100s of inks patterned automatically by incorporating a robotic fluid delivery system (38). Additionally, we currently have the ability to control 2 dimensions when fabricating these hydrogels. Future directions also include expanding into the third stage, either by including a z-stage or through grayscale patterning (39).

Despite successful demonstrations of a method that can be used to photopattern multi-domain DNA-functionalized hydrogels, lift-off via dissolution of the sacrificial layer, and collection in an unconstrained environment, limitations remain in the lift-off and collection processes. One noticeable problem in Fig 4D is the misalignment of different hydrogel domains when patterning multiple domains. This may have been caused by the weak anchoring of the hydrogel to the sacrificial layer, which causes movement of the hydrogel when ink or wash solutions are flown into the device. Sometimes the adhesion of the hydrogels to the sacrificial layer is so poor that the hydrogels patterned in one round are completely removed prematurely during subsequent patterning and wash steps, rather than during the lift-off stage. Our results indicate a relatively low yield of approximately 2% collection of 3-domain hydrogels in a single, multi-step patterning process as described in Fig 4B,C. Thus, future developments of MAPDH could be focused on building a robust sacrificial layer that can anchor photopatterned hydrogels with strong adhesion so that minimal misalignments occur during flow steps.

MAPDH could be used to help fabricate multi-domain DNA-functionalized hydrogels capable of complex shape change (40). Due to the precise control of size, location, and shape of the hydrogels, we envision that MAPDH could be used for local sequestration and release of molecules within hydrogels, either via timed release or designed reactions (6), to direct cell growth (41) or for local self-assembly (42).
Ethics declarations

If applicable

Supporting information

S1: Step-by-step protocol, also available on protocols.io

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Authors’ contributions

M.R., J.C., H.J., and R.S. wrote the paper. M.R. conceived of the idea and built the integrated platform. M.R. and J.C. and H.J. designed and performed the experiments. All authors approve the final version of the manuscript.

Metadata

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Competing interests

The authors declare no competing financial interest.

Data availability

Code and Data for this project is available at https://github.com/MishaRubanov/MAPDH.

This should include, where applicable, links to data and code produced by the protocol or necessary to interpret the outputs.

Associated content

Protocol can be found at https://dx.doi.org/10.17504/protocols.io.j8nlkw2yw5r/v1
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