SUPPORTING INFORMATION

Enzymatically Triggered Actuation of Miniaturized Tools

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Methods

Fabrication of the Grippers

The grippers were constructed on a 3 inch silicon wafer. Initially, a 30 nm chromium (Cr) adhesion layer and 150 nm copper (Cu) sacrificial layer were thermally evaporated onto the silicon. Next, the gripper features were defined by spincoating Microposit SC 1818 (Microchem) onto the wafer and patterning with a photomask (Fineline Imaging). Lift-off metallization was used to deposit 75 nm of Cr and 15 nm of gold (Au) via thermal evaporation. After stripping, the same mask was used to eletrodeposit 150 nm of Au. For the nickel (Ni) elements, another layer of photoresist was patterned and 1 μm of Ni was electroplated onto the wafer. Finally, the rigid segments of Au were patterned to be 4 μm thick. For the grippers with a second set of hinges, masks were modified such that no Cr was deposited on the bottom of the second hinge. A separate thermal evaporation was used to deposit 15 nm Cr and 75 nm Au on the top surface of the thin electrodeposited Au, in the location of the second hinge. In effect, the Cr tension was utilized on the upper Au surface, rather than the lower surface. All thermal evaporations were conducted at 10^{-5} torr.

Synthesis of Modified Polymers

Gelatin: One gram of gelatin (Type B, Sigma) was dissolved in 10 ml of 3% NaCl in deionized water. This was stirred at ~65 °C until gelatin was fully dissolved, usually in several minutes. 50 μl of methacrylic anhydride (Aldrich) was then added and the solution was stirred at 65°C for another 2 hours.[1] Before crosslinking, for every 5 ml of methacrylated gelatin, 5 ml of 1.4 w/v N,N'-Methylenebisacrylamide (BIS) (Aldrich) in deionized water was added, as well as 1.0 ml of 33% w/w Irgacure 2959 (Ciba) in ethanol.

Carboxymethylcellulose (CMC): CMC was modified via glycidyl methacrylate using an amine catalyst and phase transfer catalyst. For a batch size of 100 mL total water, a solution of 8% w/w low viscosity CMC (Sigma) was prepared by mixing and allowed to rest for several days. 12.5 mL of this solution was placed in an Erlenmeyer flask and 83.9 mL of deionized water was added. Under constant stirring, 3.6 mL of triethanolamine, 7.2 mL of 50% w/v tetra-n-butylammonium bromide (Sigma), and 3.6 mL of glycidyl methacrylate were added at 10 minute intervals. The solution was left stirring at room temperature for 24 hours, at which point the temperature was ramped to 60 ºC for one hour. The solution was then allowed to cool to room temperature for a minimum of 2 hours. The reaction mixture was carefully poured into a large evaporating dish preloaded with 2L of acetone, and the polymer precipitated out. This mixture was agitated by spinning at 100 rpm for several hours and then allowed to settle overnight. The resulting precipitate was captured by filtering through a coarse-frit funnel via gravity, and then scraped off and dried in a dish. This process was adapted from work with hylanuronic acid.[2]

For patterning, we added 1 ml of water, followed by 48 mg BIS dissolved in 1 mL of 1.5% NaCl in deionized water, to 0.1g of the modified CMC. This was allowed to vortex for several hours until it was fully dissolved. Finally, 0.20 ml of Irgacure 2959 (33% w/w in ethanol) was added. The resulting solution was filtered though a 0.4 μm syringe filter to remove stray fibers from prior collection. Osmotic swelling of the biopolymers decreased yields due to poor adhesion; this was mitigated by conducting photopatterning in a 1.5% solution of sodium chloride.

Polymerization of Polymers

Single actuation, one biopolymer, (closing action only): Quartz masks with Cr features were fabricated; Cr was left only in areas where no polymer was desired. In order to protect the mask features and decrease the adhesion of biopolymer to the mask, either a layer of polystyrene film (Trycite, 2 mil, Dow) or a spincoated perfluorinated coating (Cytop, Ashai Glass) was applied to the glass and Cr. A specific height of polymer was ensured during polymerization by outlining the wafer with polyimide tape. The tape was 76.2 μm thick, and anywhere from 1 to 5 layers were used. The gelatin was first gently heated at approximately 75 °C for 5 minutes to liquefy in order to be more easily handled. It was then placed within the area outlined by the polyimide tape. The gelatin or CMC was exposed to UV light at approximately 1000 mW/cm^2 for gelatin and 775 mW/cm^2 for CMC. The wafer was then placed in a 3% NaCl solution at room temperature to remove CMC that did not polymerize, or a heated solution to remove any of the gelatin that may have gelled but did not crosslink.

Two stage actuation, two biopolymers on one gripper, (closing and re-opening actions): After deposition of metallic layers, a 1.3 μm layer of novolac resin polymer was coated on areas not to be covered with biopolymer and used as a negative mask. This mask prevented adhesion of the biopolymer in areas where it was not desired. We used S1813 (Rohm & Haas), spun at 3000 rpm. Gelatin was initially polymerized in the second hinge region with a 10-25 μm overhang in each direction. To control the height of the gelatin one layer of polyimide tape was used to outline the wafer. The gelatin solution was then spread onto the wafer. The gelatin was then polymerized through a Cr on quartz mask, and gently heated in a 3% salt water bath a ~75 °C to remove any gelatin that had not polymerized. Following this, the wafer was partly dried with N_2

gas and another layer of polyimide tape was added to the existing tape. The modified CMC solution was then added onto the wafer and was polymerized using a different quartz mask. The wafer was placed in a 3% salt solution to rinse any CMC that had not polymerized, and then the grippers were lifted off.

Release of grippers from the substrate

The sacrificial layer was made up of a thin layer of Cr and a layer of Cu. To lift off the structures the wafer was placed in a solution of 3% NaCl. The Cu corroded away in 12-48 hrs, freeing the structures.

Degradation & Kinetics

Enzymatic triggering was studied in a 48 well plate. Each gripper was rinsed in phosphate buffered saline (PBS) and placed in a well with 0.6 mL of test liquid.

Grippers were imaged at intervals depending on closing times that were verified in preliminary experiments, the minimum time increment was 1 minute.

Details of the testing solutions:

Trypsin-EDTA (Sigma) – used as received, 25 mg/ml.

Viscozyme L (Sigma) - used as received.

Papain – (Sigma, crude latex, 1.5-10 units/mg solid) - used at 20 mg/mL, filtered at 0.22 μm.

Cellulase – (Carolina Biological Supply, 75,000 cellulase units per gram, diluted with maltodextrin), used at 22 mg/mL, filtered at 0.22 μm.

Collagenase (Worthington Biochemical Corp., 180 Units/mg) – used at 5 mg/mL, filtered at 0.22 μm.

Phosphate Buffered Saline (Gibco no added minerals) - used as received.

Cell Media: Minimum Essential Medium Eagles (Sigma) containing L-glutamine and sodium bicarbonate with 10% fetal bovine serum, supplemented with MEM nonessential amino acids and sodium pyruvate.

In vitro simulated liver biopsy

A biliary system model was prepared featuring the duodenum, pancreas, gallbladder, and liver, with a 5 mm wide common bile duct and 1 mm to 2 mm wide bile duct network. The model was cast in acrylic resin and backed with laminated paper to color various organs. Chicken liver tissue (Perdue Farms) was placed at the deep end of the bile ducts. The model's channels were filled with saline and grippers were placed in the duodenum. A magnetic stylus was used to guide the grippers to the tissue, at which point a cellulase solution (0.4 Units / 10 mL) was added to the saline. The grippers closed in under 30 minutes, and the stylus was used to extract them from the tissue and guide them back to the duodenum. Grippers were then extracted via pipette and placed in a 0.4% w/w solution of Trypan Blue stain (Sigma) for 10 minutes. After rinsing, the resulting tissue was imaged.

Cancer Cell Biopsy & RNA Extraction

The Human intrahepatic cholangiocarcinoma (HuCCT1) cell line was maintained in Dulbelcco's Modified Eagle Media (DMEM), 10% fetal calf serum (FCS), 1000 Units/mL penicillin/streptomycin in a humidified incubator at 37 °C and 5% CO_2 as previously described.[3] H69 cells (a gift from Dr. D. Jefferson at Tufts University, Boston, MA), are normal human intrahepatic cholangiocytes transformed with Simian vacuolating virus 40 (SV-40). Cells were

derived from a healthy liver prior to liver transplantation and maintained as previously described.[4]

Survival of HuCCT1 and H69 cells was tested in a medium enriched in cellulase. Varying concentrations of cellulase were tested in order to determine sensitivity: Serial dilutions from 67,500 Units/mL to 9.7 Units/mL. In terms of cell growth, cell morphology and cell count, cells were unaffected by the cellulase at all concentrations.

The ability of the grippers to collect cells was tested in HuCCT1 (grown to 80% confluence in 75 cm^2 tissue culture flasks). At this confluence the flasks contain approximately 4 million cells. H69 cells were grown in 75 cm^2 tissue culture flasks to 80% confluence. At this confluence, the flasks contain approximately 6 million cells. HuCCT1 as well as H69 cells were processed similarly, as described below.

Cells were washed twice with ice cold PBS. Two mL of Trypsin were then gently pipetted into the flasks and then the flasks were placed at 37 °C in the incubator for 5 minutes (H69 cells) or 10 minutes (HuCCT1 cells). The cells detached from the flasks and were washed by adding 5 mL of PBS per flask. The cells were then removed from the flask and centrifuged at 1500 rotations per minute (RPM). The supernatant was removed and the cells were resuspended in 2 mL of PBS. The cells were centrifuged again at 1500 RPMs in 15 mL round bottomed tubes, which formed a pellet at the bottom of the tube. The tube was tilted on its side, and 5 mL of PBS was added. Ten grippers were retrieved from their prep container with a pipette and placed at the open end of the tube. They were guided magnetically from outside the tube towards the cell pellet. The liquid was withdrawn and replaced with a 7500 U/mL solution of cellulase in PBS, which allowed sufficient working time to position the grippers and embed them into the cell pellet with magnetic force. After 20 minutes, the grippers closed, and external magnetic force

was used to dislodge a cell mass and drag it up the tube. The grippers were able to split the HuCCT1 pellet and the insertion/retrieval was repeated to extract the entire mass. The H69 cells were retrieved in one step as the grippers were sufficiently embedded to extract all of the cells in one motion. The force on these grippers was sufficient to hold the cell pellet against gravity when a rare earth magnet was applied from above. After guiding the cell mass up the tube it was retrieved via pipette.

The RNA was then extracted from the cells collected with the grippers. One mL of TRIZOL (Invitrogen) was added to cells in an Eppendorf tube and the mixture was well vortexed for 30 seconds, then kept at room temperature (RT) for 5 minutes. 200 mL of chloroform were added to each tube, then the mixture was vortexed for 15 seconds and kept at RT for 2 min. The tubes were then centrifuged at 12,000 G for 45 min at 4 °C. The aqueous phase was carefully removed from the tubes and pipetted to new Eppendorf tubes. 500 mL of isopropanol was added per tube and then the tubes were placed at -80 °C overnight. The next day, the samples were centrifuged at 4 °C for 45 minutes at 12,000 G. Following centrifugation, the supernatant was removed and the RNA pellet was washed with 1 mL of 80% ethanol per tube. The samples were then centrifuged at 7,500 Gs for 5 min at 4 °C, the supernatant was removed and the ethanol was allowed to air dry for 3 minutes. The tubes were transferred to heatblocks at 70 °C and allowed to sit for 3 minutes. The pellet was re-dissolved in 100 microliters of diethylpyrocarbonate treated water.

To assess the RNA quality, an RNA gel was performed. In brief, 72 mL of water were mixed with 1 g of agarose and then heated until it boiled for 15 seconds. The agarose was then allowed to cool to 60 °C. Ten mL of 4-morpholinopropanesulfonic acid (MOPS) 10X, 18 mL of formaldehyde 37% and 10 μL of SybrGreen (Applied Biosystems) were then added and mixed

well. The gel was poured into a gel cassette and allowed to form. The gel was then covered with MOPS running buffer 1X. Five μL of RNA per sample were mixed with 5 μL of RNA loading dye (Ambion) and loaded into the gel. The samples were run for one hour at 100 V. The images were then collected and the 18S and 28S fractions were analyzed for each sample. As a control, we used RNA extracted previously from a cell line. The quality of the control cell line was previously verified with a Bioanalyzer.

To verify the quality of the RNA from cells retrieved by the grippers, we employed electrophoresis on a denaturing agarose gel. The denaturing gel system was used because most RNA forms secondary structures via base pairing, and this might prevent it from migrating strictly according to size. To verify that the bands obtained are of correct size, we also included a positive control RNA on the gel, obtained from H69 cells not retrieved with the grippers. The upper band is 28S and the lower band is 18S (labeled in Figure 4h).

In Figure S4, the first lane is the control RNA. Lanes 2-7 represent RNA extracted from HuCCT1 cells treated with Interleukin-6 for 24 and 48 hours, respectively, for another series of experiments. Lanes 8 and 9 represent the RNA extracted from HuCCT1 cells, in duplicate. Lane 10 is RNA extracted from H69 cells. The 18S and 28S fractions are more abundant in H69 cells because there were more cells in each pellet and consequently the RNA quantity was greater. The intensity of the bands is proportional to the concentration of RNA, which results in higher intensity in lane 4 as compared to lanes 2 and 3.The figure demonstrates that the RNA obtained from cells retrieved with the grippers is of good quality, as evidenced by sharp bands of correct size (28S and 18S).

Notes on *in-vivo* Enzyme Concentrations

There are insignificant cellulases native to mammalian systems, as they are chiefly produced by single celled organisms such as bacteria and fungi (such as the bacteria in ruminants' stomachs).

The concentration of proteolytic enzymes that will degrade gelatin varies considerably *in vivo*. These enzymes are present at very high levels in the digestive tract. As an example, patients who must take replacement enzyme supplements for lack of natural pancreatic enzymes often take 100,000 units or more with each meal, well above the concentration required to actuate our gelatin grippers.

On the other hand, collagenase also describes a specific matrix metalloprotease (MMP) system, which is present at small concentrations in blood serum (clinical test kits have ranges of several ng/mL), but may be present at much higher concentrations in active spreading tumors or remodeling tissue. Concentration in the body is a function of the biological space under consideration, which may be high in confined spaces, small organelles, or near a growing tumor. In addition, total protease activity is the sum of all soluble and membrane bound proteases, which is higher than the ng/L value for a single isoform as tested by these kits.

It should be noted that our grippers are small and it is difficult to find quantified data for local concentrations of MMPs, in a size scale of the grippers (150 micron to 1mm), especially in disease states at the tissue level. A relevant value would be the concentration on first 500 microns on the surface of a growing solid tumor, for example.

The ng/mL concentration for serum is lower than the concentrations required to trigger the gelatin gripper in its present form. Further optimization will be necessary if triggering is

required at such low concentration levels *in vivo*. We expect this to be mainly in terms of reducing the thickness and modulus of the trigger layers to enable higher sensitivity.

Capture and Release of Alginate Bead

We used a two stage gripper to capture an alginate bead loaded with prussian blue pigment for visualization. The first trigger, cellulase, was introduced to close the gripper after it was positioned next to the bead. The captured bead was pipetted into the duodenum region of the cast model, and magnetically guided into the biliary tree as above. Collagenase solution was applied, which triggered the second set of hinges and re-opened the gripper. Via fluid flow and shaking with the magnetic stylus the bead was released. The bead was typically prepared by mixing 1 mL of 10% dispersion of Prussian Blue (Gamblin) in water with 5 mL of 2% alginic acid in water. After thorough mixing the resulting solution was carefully dropped into a 0.25 M solution of calcium chloride via a 30G needle. Resulting beads ranged from 400 μm to 1.5 mm in diameter.

Imaging

Microscopy imaging was performed with a Nikon AZ100 microscope with DS-Fi1 camera, Nikon TS100 inverted microscope with Qicam camera, or Canon S3 Digital Camera with macro lenses. Z-stacking was performed with NIS Elements software.

Thin Film Modeling

Multilayer thin film modeling was used to simulate a 2D cross section of the gripper from a flat state to a closed state (Figure 2). The model simulated a series of rigid segments connected by flexible hinges. Model output consists of start and end points for each component on Cartesian coordinates. Rigid segments were assumed to remain straight, and hinges were governed by the

multilayer thin film multilayer beam bending equations as described previously.[5-7] Relevant parameters were material thicknesses: 75 nm Cr, 150 nm Au, 150 μm biopolymers, initial stress: 0.8 GPa for Cr, and elastic moduli: 144 GPa for Cr and 78 GPa for Au.[8] For each connected region a start point, end, point, and angle change were calculated. Vector calculations were used to add these in series and plot points and lines to visualize the gripper. The modulus of the biopolymer layer was then lowered in the simulation and varying tip-to-tip distance was calculated.[9]

This model was extended to grippers with a second hinge that closed and re-opened by again modeling all rigid segments and flexible hinges (17 elements for a cross-section of this design). We extended the model by incorporating the two hinge types (concave up or concave down), and the simulation was modified to direct the second hinge in an opposite direction.[10, 11] Each polymer could be modeled independently.

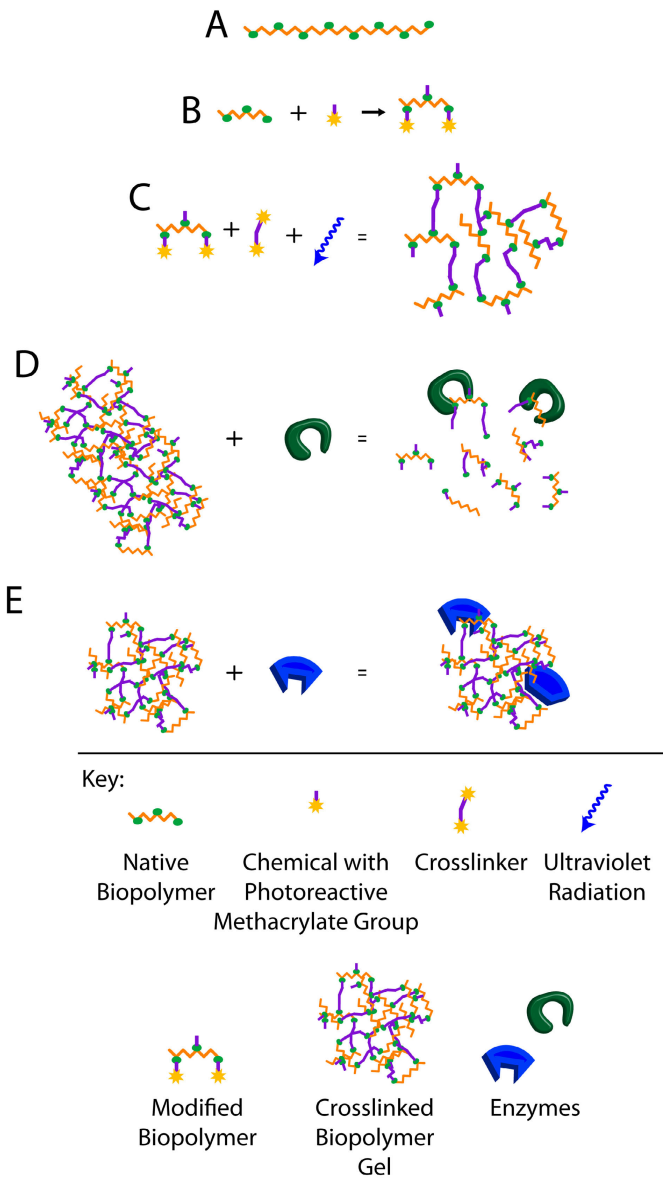

Figure S1. Schematic for biopolymer patterning and degradation. A schematic for biopolymer modification, patterning, and degradation. (**A**) Shows the native biopolymer with enzymatic recognition sites. (**B**) This biopolymer is grafted with photoreactive groups via chemical reaction. Next, the modified biopolymer is combined with crosslinker, photoinitiator and patterned with UV light (**C**). This biopolymer is degraded only by appropriate enzymes (**D**). Other enzymes will diffuse into the hydrogel, but do not degrade the crosslinked biopolymer (**E**).

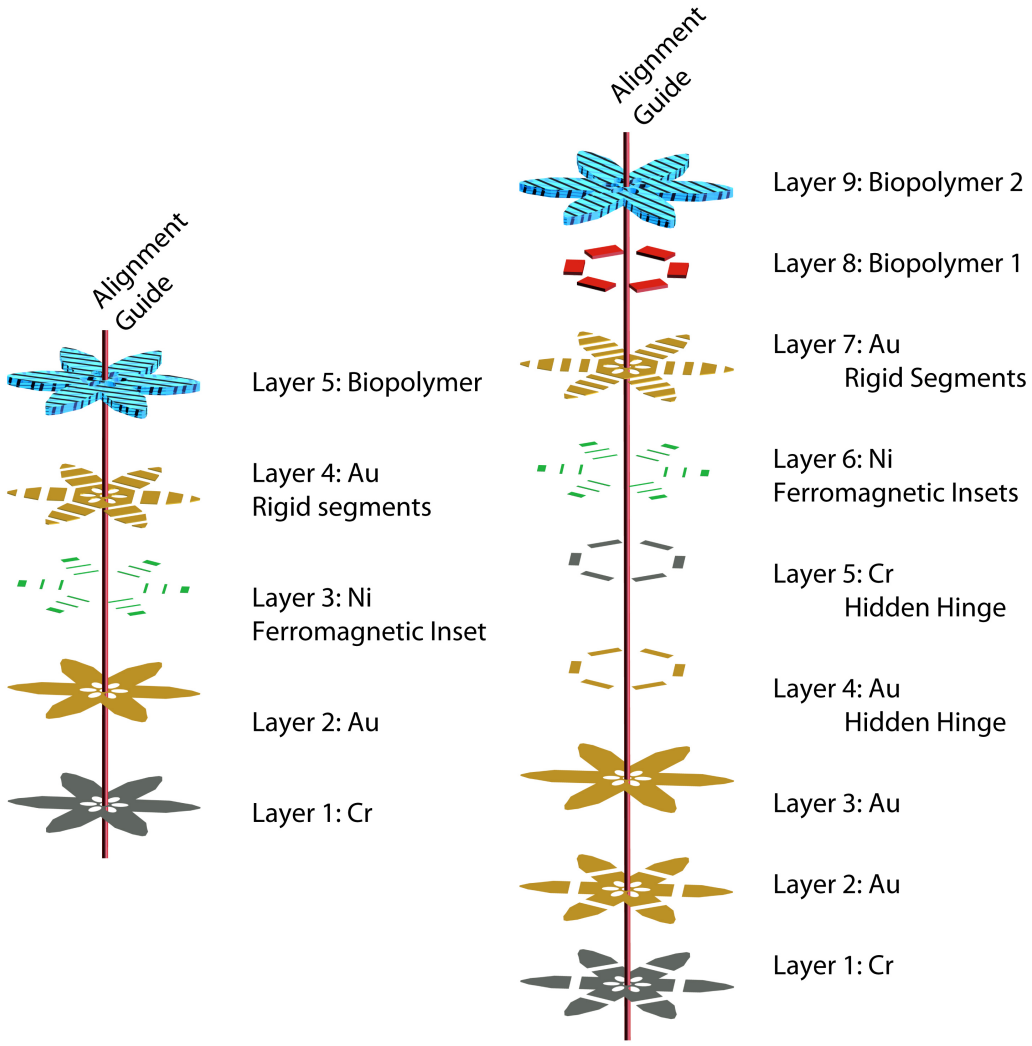

Figure S2. Schematic for biopolymer patterning and degradation. Process flow of the important steps in the fabrication sequence (top to bottom) for the grippers. Metal layers were deposited via thermal evaporation (Cr, Au) and electrodeposition (Au, Ni). Biopolymers were patterned directly via UV photocrosslinking as a final step. Only the Cr layers exhibited stress and drove closing or re-opening, while the polymer layers were stiff as patterned and prevented bending. Ferromagnetic Ni layers allowed for guidance with an external magnet.

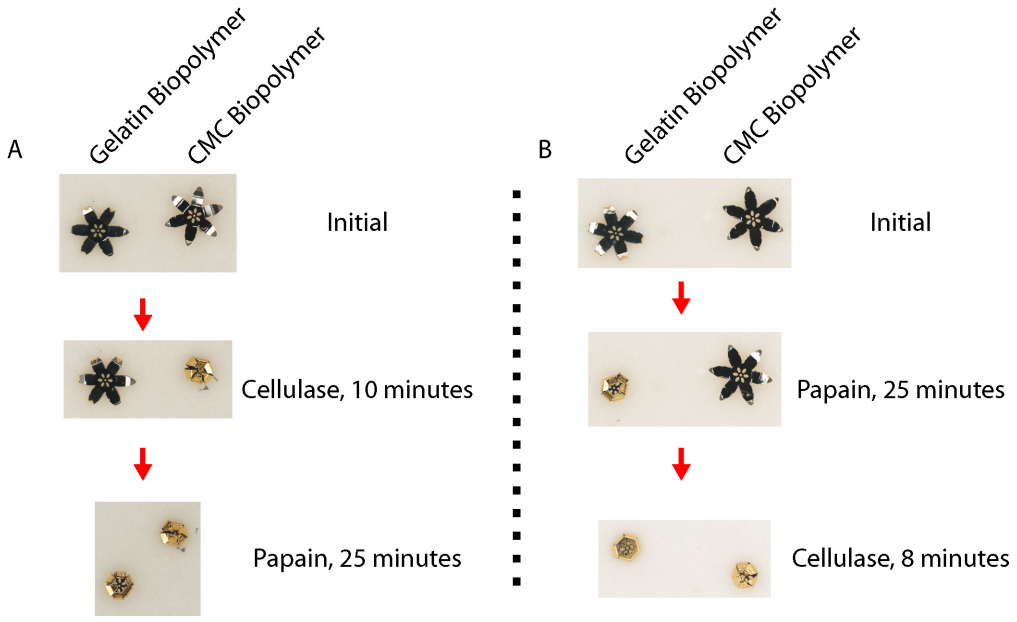

Figure S3. Grippers can be closed specifically by biopolymer hinge type. (**A**) Gelatin and CMC grippers show specificity, actuating only in the presence of protease enzymes and polysaccharidases, respectively. When grippers with both types of biopolymer are added into the protease, only the gelatin grippers close. When the protease is removed and polysaccharidases are added, the CMC grippers then close. The converse results are demonstrated when the order of the enzymes added is reversed in (**B**).

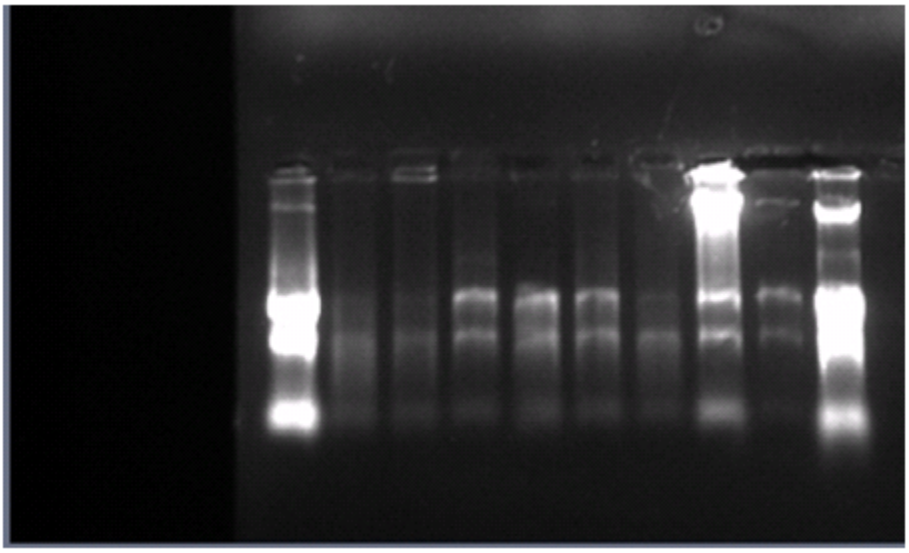

Figure S4. RNA Extraction from captured cells. The original scan of the RNA gel shown cropped in Figure 4 of the main text. Leftmost lane is the control, rightmost 3 lanes are H69 cells retrieval 1, H69 cells retrieval 2, and HuCCT1 cell retrieval.

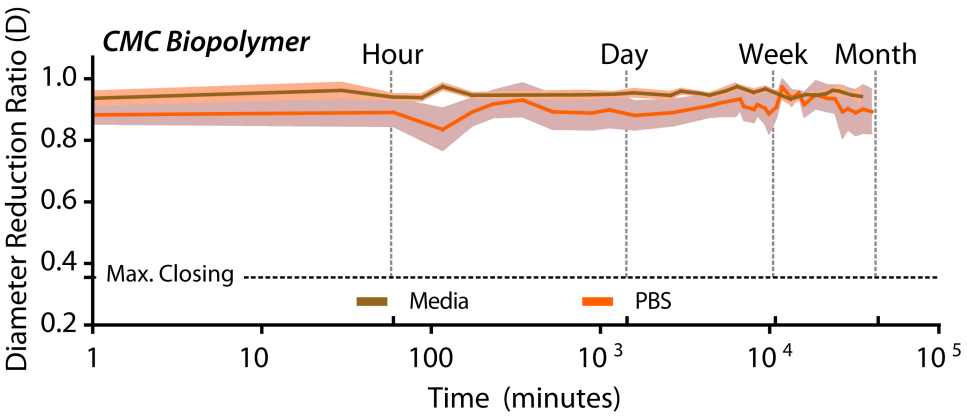

Figure S5. CMC grippers in PBS and Cell Media. Additional data from Figure 3 in the main text. Biopolymer grippers were placed in microwells and control solutions added. The CMC biopolymer grippers did not close in either PBS or cell culture media with serum for a period of a month.

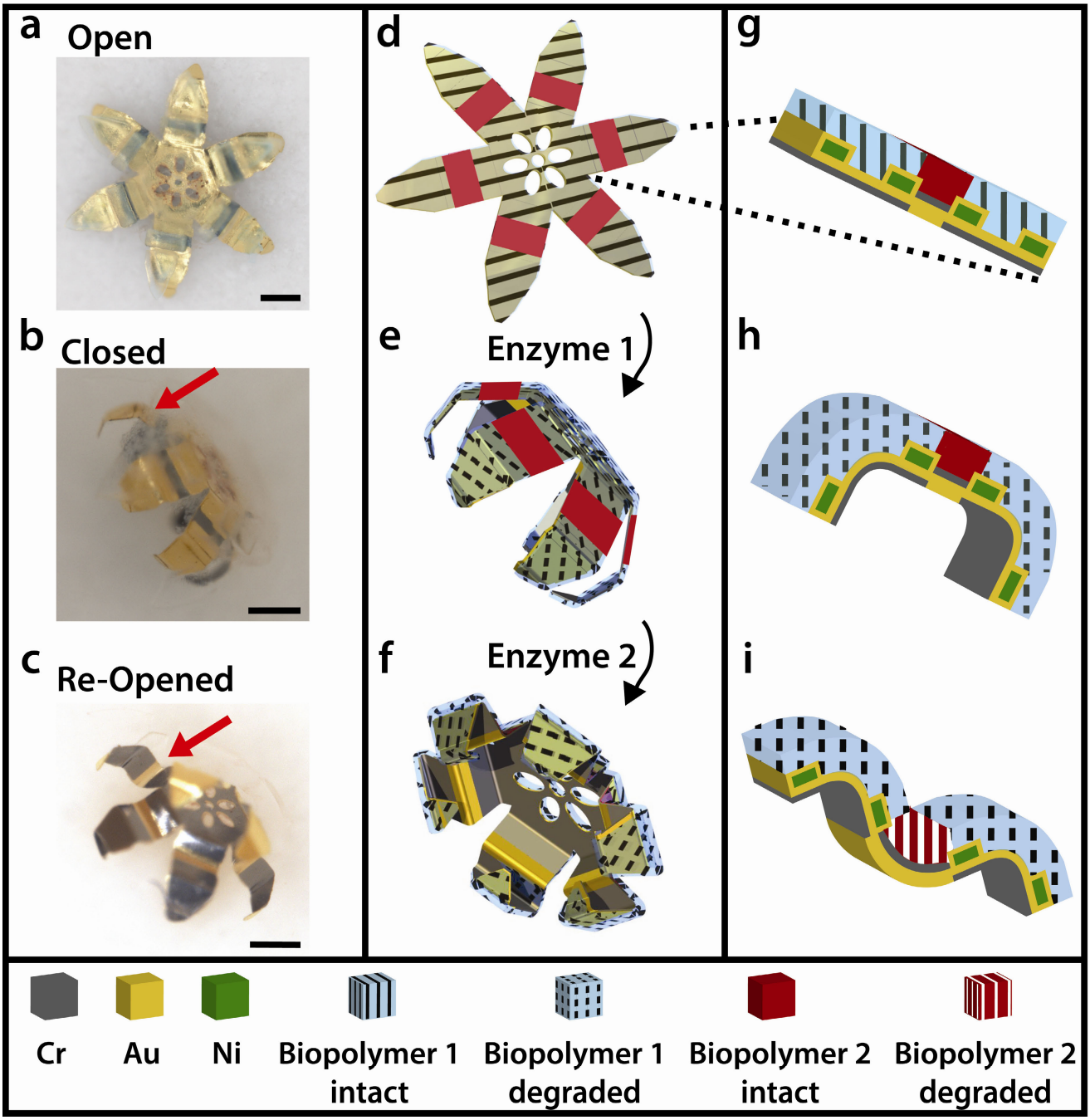

Figure S6. A larger reproduction of Figure 1 in the text is provided for ease of viewing.

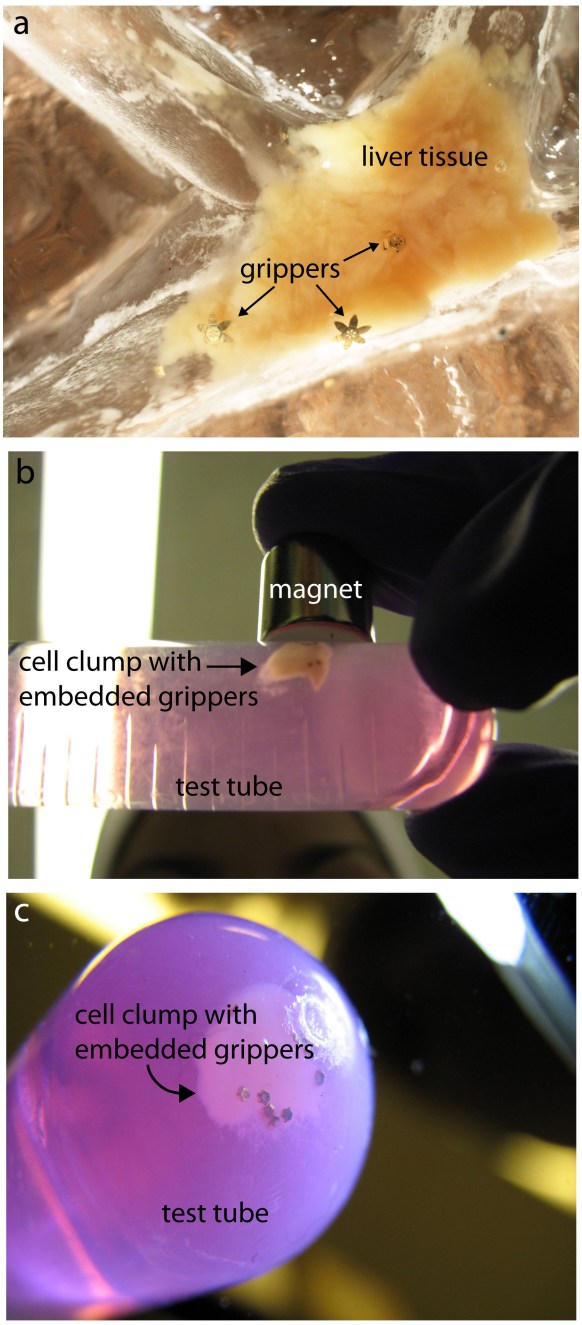

Figure S7. Larger views of panels in Figure 4. (A) demonstrates the microgripper biopsy experiment in the acrylic liver model shown in panel 4d. (B) shows the entire image from which panel 4f was cropped, with the magnet holding microgrippers in a cell clump against gravity. (C) shows an image from which panel 4g was cropped, with actuated microgrippers in the cell clump.

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