

# Tetherless thermobiochemically actuated microgrippers

Timothy G. Leong<sup>a</sup>, Christina L. Randall<sup>b</sup>, Bryan R. Benson<sup>a</sup>, Noy Bassik<sup>a</sup>, George M. Stern<sup>a</sup>, and David H. Gracias<sup>a,c,1</sup>

Departments of <sup>a</sup>Chemical and Biomolecular Engineering and <sup>c</sup>Chemistry, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218; and <sup>b</sup>Department of Biomedical Engineering, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Baltimore, MD 21205

Edited by James R. Heath, California Institute of Technology, Pasadena, CA, and accepted by the Editorial Board November 25, 2008 (received for review August 5, 2008)

**We demonstrate mass-producible, tetherless microgrippers that can be remotely triggered by temperature and chemicals under biologically relevant conditions. The microgrippers use a self-contained actuation response, obviating the need for external tethers in operation. The grippers can be actuated en masse, even while spatially separated. We used the microgrippers to perform diverse functions, such as picking up a bead on a substrate and the removal of cells from tissue embedded at the end of a capillary (an in vitro biopsy).**

actuator | biochemical | robotics | thin films

**B**iological function in nature is often achieved by autonomous organisms and cellular components triggered en masse by relatively benign cues, such as small temperature changes and biochemicals. These cues activate a particular response, even among large populations of spatially separated biological components. Chemically triggered activity is also often highly specific and selective in biological machinery. Additionally, mobility of autonomous biological entities, such as pathogens and cells, enables easy passage through narrow conduits and interstitial spaces.

As a step toward the construction of autonomous microtools, we describe mass-producible, mobile, thermobiochemically actuated microgrippers. The microgrippers can be remotely actuated when exposed to temperatures  $>40^{\circ}\text{C}$  or selected chemicals. The temperature trigger is in the range experienced by the human body at the onset of a moderate-to-high fever, and the chemical triggers include biologically benign reagents, such as cell media. Using these microgrippers, we achieved a diverse set of functions, such as picking up beads off substrates and removing cells from tissue samples.

Conventional microgrippers are usually tethered and actuated by mechanical or electrical signals (1–6). Recently developed actuation mechanisms using pneumatic (7), thermal (8), and electrochemical triggers (9, 10) have also used tethered operation. Because the functional response of currently available microgrippers is usually controlled through external wires or tubes, direct connections need to be made between the gripper and the control unit. These connections restrict device miniaturization and maneuverability. For example, a simple task such as the retrieval of an object from a tube is challenging at the millimeter and submillimeter scale, because tethered microgrippers must be threaded through the tube. Moreover, many of the schemes used to drive actuation in microscale tools use biologically incompatible cues, such as high temperature or nonaqueous media, which limit their utility. There are novel, untethered tools based on shape memory alloys that use low temperature heating, but they have limited mobility and must rely solely on thermal actuation (11, 12). The ability of our gripper design to use biochemical actuation, in addition to thermal actuation, represents a paradigm shift in engineering and suggests a strategy for designing mobile microtools that function in a variety of environments with high specificity and selectivity.

To engineer untethered, mobile grippers, we developed an actuation mechanism that used trilayer joints composed of a polymer and a stressed bimetallic thin film patterned between rigid phalanges (Fig. 1). The microgrippers were fabricated by using conventional multilayer photolithography on a water-soluble sacrificial polyvinyl alcohol layer; this allowed open grippers to be released from the substrate in water. Briefly (detailed in *Methods*), chromium (Cr) and copper (Cu) thin films were thermally evaporated onto the sacrificial layer that had been spin-coated on a silicon wafer. Then, 2 steps of photolithography were performed to fabricate the microgrippers: The first step patterned nickel (Ni) and gold (Au) phalanges, and the second step patterned the polymer trigger and bimetallic (Cr/Cu) layer of the joint. This process enabled large numbers of grippers to be fabricated in a highly parallel and cost-effective manner.

The grippers were structured on a hierarchy of length scales; the films driving actuation were 50–300 nm thick, and the phalanges and joints were tens to hundreds of microns in width and  $\approx 7\ \mu\text{m}$  thick. The entire gripper system was self-contained with a size as small as 700  $\mu\text{m}$  when open and 190  $\mu\text{m}$  when closed. The overall shape of the microgrippers was modeled after biological appendages, such as hands, in which the jointed digits are arranged in different ways around a central palm. For example, the digits in the human hand are arranged in a rotationally asymmetric manner and contain a varying amount of joints; 4 digits contain 3 interphalangeal joints, whereas the fifth (thumb) only has 2 (13). In our gripper designs, we varied the number and arrangement of digits around the palm (Fig. 2*A* and *B*), the shape of the central polygonal palm (Fig. 2*C–E*), and the number of interphalangeal joints (Fig. 2*F–H*). We also incorporated tapered distal phalanges (emulating sharp nails or claws) to enable extrication of cells and tissue in our experiments.

We observed that an asymmetric arrangement of digits left a large gap within the closed gripper (Fig. 2*A*), whereas grippers with a symmetrical arrangement of digits (Fig. 2*B*) retained objects more effectively. We found that grippers with fewer digits were more likely to close properly (higher yield), because a smaller number of joints needed to flex. However, grippers with more digits had an increased defect tolerance and were still able to hold on to objects despite a few defective joints. In our study, we found that grippers with 6 rotationally symmetric digits achieved a sufficient balance between yield and defect tolerance.

Author contributions: T.G.L., C.L.R., B.R.B., and D.H.G. designed research; T.G.L., C.L.R., B.R.B., and G.M.S. performed research; N.B. and G.M.S. contributed new reagents/analytic tools; T.G.L., C.L.R., N.B., G.M.S., and D.H.G. analyzed data; and T.G.L., C.L.R., B.R.B., N.B., G.M.S., and D.H.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. J.R.H. is a guest editor invited by the Editorial Board.

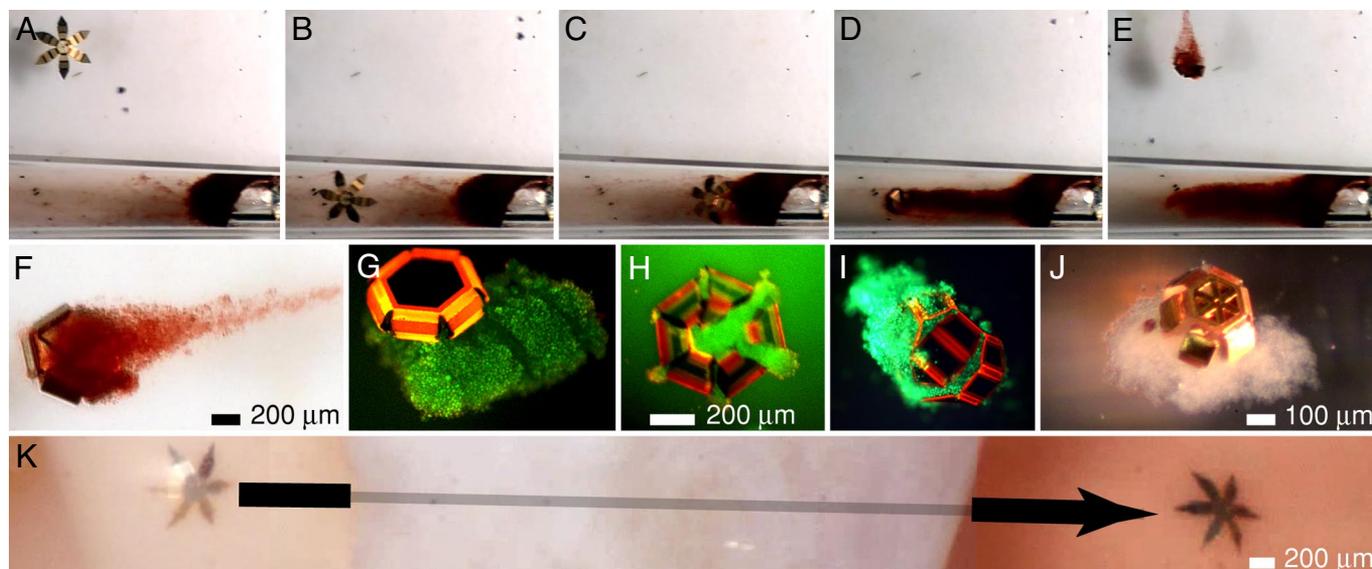
<sup>1</sup>To whom correspondence should be addressed at: 3400 North Charles Street, 125 Maryland Hall, Baltimore, MD 21218. E-mail: dgracias@jhu.edu.

This article contains supporting information online at [www.pnas.org/cgi/content/full/0807698106/DCSupplemental](http://www.pnas.org/cgi/content/full/0807698106/DCSupplemental).

© 2009 by The National Academy of Sciences of the USA







**Fig. 5.** Thermally and biochemically triggered cell capture. (A–E) Optical microscopy sequence showing the thermally triggered capture and retrieval of Neutral red-stained cells from a cell culture mass at the end of a 1.5-mm-diameter tube (Movie S3). (F) Zoomed detail of the microgripper with the cells captured in A–E demonstrating viability (red). (G) Fluorescent micrograph demonstrating viability of thermally captured LIVE/DEAD stained cells. Note that the photopatternable polymer in the joints fluoresces red under UV excitation. (H) Fluorescent micrograph with viable cells (green) captured by using a thermal trigger and incubated for 72 h afterward. (I) Fluorescent micrograph of viable cells captured by using a biochemical trigger to actuate the gripper. (J) Optical image of a microgripper with captured cells from a sample of a bovine bladder. (K) Overlaid optical micrograph sequence depicting the traversing of a gripper from left to right through an orifice in a bovine bladder tissue sample.

into a capillary tube and thermally triggered to grasp a portion of a living cell mass stained with Neutral red (a red stain that accumulates in lysosomes after diffusing through the cell membrane of viable cells). The microgripper was then guided out of the capillary with the captured cells in its grasp (Fig. 5D and E). The cells were still viable, as indicated by their red color, and the cluster of cells after retrieval can be seen in Fig. 5F. The experiment was repeated numerous times with LIVE/DEAD stain to further demonstrate cell viability after thermally triggered capture (Fig. 5G). After retrieval, we placed cell-loaded grippers into media and incubated them for 72 h; the cells were still viable (Fig. 5H), signifying that the materials used in the fabrication of the grippers and the capture and retrieval processes were not harmful to the cells. The viability of the captured cells after incubation provides evidence that the grippers could be used as biological storage devices until the samples are ready to be analyzed.

In addition to thermally triggering the grippers, we used biochemical-triggered actuation to capture live cells. We observed that grippers closed to some degree in a variety of biochemicals, including aqueous solutions of glucose, trypsin, and L929 cell media (detailed list in SI). It is important to note that the time required to close the microgrippers with biochemical triggers was longer than that needed with thermal or caustic/solvent-based actuation. We believe that biochemical actuation occurs as a result of a chemical attack of the polymer–Cu interface, a process that takes longer and results in decreased adhesion of the polymer. Based on the results from our biochemical screening and the fact we were using L929 cells, we chose to use L929 cell media as a biocompatible trigger. Grippers were placed into centrifuged solutions containing L929 cells and L929 media and then placed into an incubator to sustain the cells. It is important to note that the grippers partially closed at room temperature when exposed to L929 media but further closed under the incubator conditions used to maintain the cells (details in Table S1). After 30 min, the grippers closed around cells, and they were imaged after 4 h. Rather than imaging the

grippers immediately, waiting 4 h allowed us to determine whether any apoptotic (as opposed to necrotic) cell death had occurred. Fig. 5I features a fluorescent micrograph of a gripper closed around live (green) L929 cells after 4 h in L929 media.

To explore the utility of the gripper in microsurgical applications, we performed an in vitro biopsy on a tissue sample from a bovine bladder (Fig. 5J) loaded into a 1.5-mm glass capillary tube (see also Fig. S3). This experiment necessitated the use of the magnet to rotate the gripper such that the claw phalanges could cut through the connective tissue and extricate the cells. This experiment demonstrated that even though the grippers had nanomicroscale actuation joints, they were strong enough to perform an in vitro tissue biopsy.

Also, we magnetically manipulated a microgripper in different regions of whole bovine bladder tissue, including both rough and smooth areas. It was observed that the claw would become adhered and immobile when manipulated across the rough tissue, because the claw phalanges became entangled with the tissue. However, the gripper could eventually be dislodged with continued magnetic manipulation. On smooth tissue, it was observed that the claw could be easily manipulated. Fig. 5K shows a gripper that was manipulated through an orifice in the bladder tissue.

Once closed, the contents of the grippers could be retrieved in a biocompatible manner by mechanical disruption. Beads retrieved from the gripper were not damaged, and retrieved cells were viable (Figs. S2 and S4). After retrieving the microgrippers with captured cells, they were mechanically agitated by using a variety of methods (including force applied via Pasteur pipette tip, prying open with 22-G syringe tips, and vortexing) to release the cells. Vortexing also provided a way of releasing the cells and leaving the microgripper intact. Vortexing dispersed the cells, making them hard to find and image, but the cells could be centrifuged and collected for further testing. To release captured clumps of cells without dispersing them, we used either a Pasteur pipette or syringe tips to open the grippers; however, this method typically damaged the microgrippers. Ideally, it would be pos-



to settle due to gravity as a large cell mass. The loaded capillary was placed into a Petri dish filled with L929 cell media and a microgripper. The microgripper was magnetically manipulated into the capillary and captured a clump of the cells upon heating. This process was easily performed and successfully repeated >2 dozen times. Upon retrieval from the capillary, the microgripper loaded with captured fibroblast cells was placed into new media and incubated for 72 h. LIVE/DEAD stain was applied to verify the viability of the cells.

**Biochemical-Triggered Capture of Cells: Experimental Details of the Demonstration Shown in Fig. 5J.** Two separate tests were devised to capture live cells by using biochemically induced actuation. In the first test, L929 cells were centrifuged, and half of the supernatant was added to a small plastic Petri dish containing 5 mL of L929 media. Two grippers were then added to the Petri dish, and the dish was placed into an incubator at 37 °C. In the second test, cells were centrifuged in L929 media, and 2 grippers were added directly to the centrifuge tube, where they settled into the cell mass. The test tube was incubated at 37 °C. The grippers were separated from the cells by using a magnet and imaged after 4 h.

**In Vitro Biopsy of a Bovine Bladder: Experimental Details of the Demonstration Shown in Fig. 5J and Fig. 53.** A core sample of bovine bladder tissue (Innovative Research) was taken with a 1.5-mm-diameter glass capillary, thus plugging the end of the capillary tube. The experiment was performed in a manner similar

to the cell-capture experiments. A microgripper was guided into the capillary and heated to close around a clump of the bladder tissue. To retrieve the microgripper with a sample, the magnet used for guidance was rotated to spin the microgripper, allowing the nail phalanges to cut the connective tissue, extricate the cell mass, and become free of the tissue. After cutting through, the microgripper was guided out of the capillary and imaged with the captured bladder cells.

**Manipulation of a Gripper in Bovine Bladder Tissue (Fig. 5K).** Bovine bladder tissue was placed into a large Petri dish filled with phosphate buffer saline. A gripper was placed into the solution on the bladder tissue. We manipulated the gripper through an opening in the tissue where blind manipulation was required.

**ACKNOWLEDGMENTS.** We acknowledge the illustration contributions from Aasiyeh Zarafshar and Anum Azam and differential scanning calorimetry assistance from Kedar D. Deshmukh and Howard E. Katz. This material is based in part on work supported by the National Science Foundation under Grants CMMI-0448816 and DMR05-20491; by the National Institutes of Health under Grant 1R21EB007487-01A1; and by the Dreyfus and the Beckman Foundations. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the funding agencies.

1. Angelo JA (2006) *Robotics, A Reference Guide to a New Technology* (Greenwood Press, Westport, CT).
2. Madden JD (2007) Mobile robots: Motor challenges and materials solutions. *Science* 318:1094–1097.
3. Cecil J, Powell D, Vasquez D (2007) Assembly and manipulation of micro devices—A state of the art survey. *Robot Comput Integrated Manufacturing* 23:580–588.
4. Kim CJ, Pisano AP, Muller RS, Lim MG (1992) Polysilicon microgripper. *Sensors Actuators A Phys* 33:221–227.
5. Pister KSJ, Judy MW, Burgett SR, Fearing RS (1992) Microfabricated hinges. *Sensors Actuators A Phys* 33:249–256.
6. Lee AP, et al. (1996) A practical microgripper by fine alignment, eutectic bonding and SMA actuation. *Sensors Actuators A Phys* 54:755–759.
7. Lu YW, Kim CJ (2006) Microhand for biological applications. *Appl Phys Lett* 89:1641011–1641013.
8. Luo JK, et al. (2006) Modelling and fabrication of low operation temperature microcages with a polymer/metal/DLC trilayer structure. *Sensors Actuators A Phys* 132:346–353.
9. Jager EWH, Inganäs O, Lundström I (2000) Microrobots for micrometer-size objects in aqueous media: Potential tools for single-cell manipulation. *Science* 288:2335–2338.
10. Shahinpoor M, Bar-Cohen Y, Simpson JO, Smith J (1998) Ionic polymer-metal composites (IPMCs) as biomimetic sensors, actuators and artificial muscles—A review. *Smart Mater Struct* 7:R15–R30.
11. Buckley PR, et al. (2006) Inductively heated shape memory polymer for the magnetic actuation of medical devices. *IEEE T Biomed Eng* 53:2075–2083.
12. Small W, et al. (2007) Prototype fabrication and preliminary in vitro testing of a shape memory endovascular thrombectomy device. *IEEE T Biomed Eng* 54:1657–1666.
13. Flatt AE (2002) Our thumbs. *Proc Baylor Univ Med Center* 15:380–387.
14. Chapman RF (1982) *The Insects: Structure and Function* (Harvard Univ Press, Cambridge, MA).
15. Abermann R, Martinz HP (1984) Internal stress and structure of evaporated chromium and MgF<sub>2</sub> films and their dependence on substrate temperature. *Thin Solid Films* 115:185–194.
16. Hoffman RW, Daniels RD, Crittenden EC, Jr (1954) The cause of stress in evaporated metal films. *Proc Phys Soc London B* 67:497–500.
17. Klokholm E, Berry BS (1968) Intrinsic stress in evaporated metal films. *J Electrochem Soc* 115:823–826.
18. Thornton JA, Hoffman DW (1989) Stress-related effects in thin films. *Thin Solid Films* 171:5–31.
19. Arora WJ, Nichol AJ, Smith HI, Barbastathis G (2006) Membrane folding to achieve three-dimensional nanostructures: Nanopatterned silicon nitride folded with stressed chromium hinges. *Appl Phys Lett* 88:0531081–0531083.
20. Chua CL, Fork DK, Van Schuylenbergh K, Lu JP (2003) Out-of-plane high-Q inductors on low-resistance silicon. *J Microelectromech Soc* 12:989–995.
21. Moiseeva E, Senouy YM, McNamara S, Harnett CK (2007) Single-mask microfabrication of three-dimensional objects from strained bimorphs. *J Micromech Microeng* 17:N63–N68.
22. Schmidt OG, Eberl K (2001) Thin solid films roll up into nanotubes. *Nature* 410:168.
23. Doerner MF, Nix WD (1988) Stresses and deformation processes in thin films on substrates. *CRC CR Rev Sol State* 14:225–268.
24. Leong TG, Benson BR, Call EK, Gracias DH (2008) Thin film stress-driven self-folding of microstructured containers. *Small* 4:1605–1609.
25. Morton SL, Degertekin FL, Khuri-Yakub BT (1999) Ultrasonic sensor for photoresist process monitoring. *IEEE Transact Semiconductor Manufacturing* 12:332–339.
26. Gogolides E, Tegou E, Beltsios K, Papadokostaki, Hatzakis M (1996) Thermal and mechanical analysis of photoresist and silylated photoresist films: Application to AZ 5214™. *Microelectron Eng* 30:267–270.
27. Paniez PJ, Chollet J-PE, Pons MJ (1993) Thermal properties of state of the art novolak-diazonaphthoquinone systems: Differences between bulk and film properties. *Proc SPIE* 1925:614–625.
28. Nikishkov GP (2003) Curvature estimation for multilayer hinged structures with initial strains. *J Appl Phys* 94:5333–5336.
29. Bassik NB, Stern GM, Gracias DH (2008) Patterning thin film mechanical properties to drive assembly of complex 3D structures. *Adv Mater* 20:4760–4764.
30. Qi ZQ, Meletis EI (2005) Mechanical and tribological behavior of nanocomposite multilayered Cr/a-C thin films. *Thin Solid Films* 479:174–181.
31. Freund LB, Suresh S (2003) *Thin film materials: Stress, Defect Formation, and Surface Evolution* (Cambridge Univ Press, Cambridge, UK).
32. Jamani KD, Harrington E, Wilson HJ (1989) Rigidity of elastomeric impression materials. *J Oral Rehab* 16:241–248.
33. Gimi B, et al. (2005) Self-assembled three dimensional radio frequency (RF) shielded containers for cell encapsulation. *Biomed Microdev* 7:341–345.
34. Ye HK, et al. (2007) Remote radio-frequency controlled nanoliter chemistry and chemical delivery on substrates. *Angew Chem Int Ed* 46:4991–4994.