Programmable assembly of pressure sensors using pattern-forming bacteria

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Biological systems can generate microstructured materials that combine organic and inorganic components and possess diverse physical and chemical properties. However, these natural processes in materials fabrication are not readily programmable. Here, we use a synthetic-biology approach to assemble patterned materials. We demonstrate programmable fabrication of three-dimensional (3D) materials by printing engineered self-patterning bacteria on permeable membranes that serve as a structural scaffold. Application of gold nanoparticles to the colonies creates hybrid organic-inorganic dome structures. The dynamics of the dome structures’ response to pressure is determined by their geometry (colony size, dome height, and pattern), which is easily modified by varying the properties of the membrane (e.g., pore size and hydrophobicity). We generate resettable pressure sensors that process signals in response to varying pressure intensity and duration.

Nature presents many forms of microstructured materials, fabricated from the bottom up, that combine living and non-living components and have advantageous physical properties. For example, mollusk shells, composed of multilayered microstructures consisting of calcium carbonate interlaced with a small amount of organic components1, are three orders of magnitude tougher than non-biogenic calcium carbonate2,3. In contrast to conventional physical and chemical synthesis methods, biological fabrication is environmentally friendly and often relies on the self-assembly of building blocks. Advances in synthetic biology and biomaterials engineering4–6 have demonstrated the self-assembly of structures from various biological building blocks7–10, including proteins7–9, peptides10–12, and DNAs13–16. Some of these structures combine organic and inorganic components. For example, the naturally occurring S-layer protein can self-assemble into different shapes such as sheets or open cylinders17 and can serve as a template for assembling cadmium sulfide (CdS) nanocrystals into a superlattice structure18–20.

Another form of hybrid organic–inorganic material uses engineering of bacteria to control the formation of biofilms that assemble inorganic components across various length scales. In a recent study, Chen et al.21 engineered bacteria to produce curli amyloid. Curli is part of the extracellular matrix produced by many bacteria and consists of two components, CsgA and CsgB22. In this system21, the timing and duration of the gene circuit inducer controlled the production and patterning of curli. The conductive biofilms generated from this inorganic–organic system combined with an electrode were externally controlled as electronic switches21. However, the assembly of nanoparticles was mediated by prepatterning of bacteria on a two-dimensional (2D) surface combined with exogenous induction of curli expression. This approach limits the tunability of the physical properties of the assembled materials.

Here, we address these limitations by assembling nanoparticles through programmed self-organized pattern formation in engineered bacteria. We achieve this by further engineering an existing synthetic gene circuit that programs bacterial pattern formation23. The circuit consists of a mutant T7 RNA polymerase (T7RNAP)24 that activates its own expression through a T7 promoter carrying an operator site (lacO) repressed by LacI, as well as that of LuxR and LuxI. LuxI synthesizes an acyl-homoserine lactone (AHL), a membrane-diffusible chemical that upon binding and activating LuxR, induces expression of T7 lysozyme, which inhibits T7RNAP25. Cyan fluorescent protein (CFP) and mCherry fluorescent protein are co-expressed with T7RNAP and lysozyme, respectively, to report the circuit dynamics. When turned on by addition of exogenous isopropyl-D-1-thiogalactopyranoside (IPTG), the circuit enables generation of robust spatial patterns in the expression of mCherry23,26.

We extended our 2D patterning circuit23 by incorporating the engineered curli developed by Chen et al.21. The bacteria we engineered produced and assembled extracellular curli fibrils with functional tags into three-dimensional (3D) patterns. These patterned curli fibrils in turn enabled assembly of inorganic materials. This two-layer control enables assembly of structured materials that have well-defined physical and chemical properties (Fig. 1), in our case, the capability to sense external pressure. A pressure sensor assembled by living cells

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results in the formation of a Ni (II)-NTA bond between the 6×-His tag and the gold gated with a Ni-nitrilotriacetic acid (NTA) group, through the for-
dependent manner (Supplementary Fig. 1a). Addition of exogenous AHL allowed activation of curli without requiring a high culture density.

These curli fibrils enabled assembly of gold nanoparticles conjugated with a Ni-nitrilotriacetic acid (NTA) group, through the forma-
tion of a Ni (II)-NTA bound between the 6×-His tag and the gold nanoparticle (Supplementary Fig. 1e). To assemble other inorganic particles, we used a mouse anti-6× His-tag-antibody-conjugated biotin, which can bind to anti-mouse antibody conjugated with nanoparticles. As a demonstration, we used goat anti-mouse IgG conjugated with 10 nm gold. Assembly of the gold particles occurred when both antibodies were present (Supplementary Fig. 1f), but not when either was absent (Supplementary Fig. 1g). By changing the conjugation module on the secondary antibody, we can assemble different inorganic nanoparticles, including CdSe quantum dots (Supplementary Fig. 1h).

Generation of tunable bacterial patterns in 3D

We used inkjet printing to initiate single colonies on permeable membranes placed on top of growth media containing agar. The membranes served as a structural support for colony growth and greatly facilitated subsequent assembly of nanoparticles. Briefly, we printed a 150-μL (containing ~20 cells) droplet of the bacterial culture onto each membrane. When confined in 2D, bacteria carrying our pattern formation circuit generated 2D patterns. Here, we did not confine the bacterial growth to enable 3D pattern formation. After a 32-h incubation at 30 °C, each colony grew into a raised, convex shape, with a 3D dome pattern of mCherry expression within (Fig. 2a and Supplementary Fig. 2a). The colony size, elevation, and the corresponding pattern were controllable by the hydrophobicity and pore size of the membrane. For a fixed pore size, the colonies became flatter with decreasing membrane hydrophobicity. If a membrane was too hydrophobic (e.g., a PTFE membrane), it prevented colony growth, likely by suppressing wicking of nutrient from the agar and making it unavailable to the bacteria. For membranes of the same material (thus with similar hydrophobicity), the colonies became larger but not taller with increasing pore sizes (Fig. 2b and Supplementary Fig. 2b).

Mechanistically, the observed dependence of colony shape on the physical properties of the membrane can be attributed to modulation of cellular motility on, and nutrient transport across, the membrane. We developed a phenomenological kinetic model that accounts for these effects to examine how colony morphology and gene expression patterns are controlled by the physical properties of the membranes. Indeed, the model captured the characteristics of the dome structure, as well as its dependence on membrane properties (Fig. 2c and Supplementary Fig. 2c). Similarly, consistent with experimental observations (Fig. 2d and Supplementary Fig. 2d), our model shows that decreasing hydrophobicity led to colonies with larger radii and lower heights (Fig. 2e and Supplementary Fig. 2e).

Assembly and evaluation of pressure sensors

We next assembled gold nanoparticles using the bacteria carrying the curli-pattern circuit. Briefly, we fixed each colony by floating the supporting membrane on high-concentration fixation solution and then immunolabeled gold nanoparticles onto the structure (Supplementary Fig. 3a). As curli and mCherry are co-expressed, we expected their spatial patterns to overlap. Therefore, the structure of gold nanoparticles assembled by curli should also overlap with that of mCherry. Indeed, confocal microscopy shows that the spatial distribution of the nanoparticles was similar to that of mCherry (Supplementary Fig. 3b), both forming a dome.

The dome is a composite material consisting of inorganic gold nanoparticles distributed in an organic matrix. As the organic matrix is visco-elastic and the gold nanoparticles are conductive, we conjectured that the assembled microstructure could serve as a pressure sensor. Consider two bacterially fabricated domes facing each other and separated by a small distance, and a constant voltage applied to the edge of each dome (Supplementary Fig. 4a). When sufficiently pressed, the two domes make contact, which leads to the flow of an electrical current. Since the interparticle distances decrease, and the number of particle-particle contacts increase, the strength of the current should reflect the strength of the externally applied pressure.

To test this notion, we used nitrocellulose to mount a nuclease track-etched polycarbonate (PC) membrane with a colony onto a thin glass coverslip. We then positioned two such glass coverslips (each carrying a colony) to face each other with a 0.5-mm-thick silicone gasket as a spacer in between (Supplementary Fig. 4b). Next, we used copper wire to connect the edges of the colonies to an electrochemistry workstation. The workstation provided a constant voltage to the device and recorded the changes in the electronic current flowing through the device. To actuate this pressure-sensitive device, we placed a thin cylinder (~1.5 mm in diameter) right on top of the upper glass coverslip. The center axis of the cylinder was aligned with the center of the two colonies. The device was then actuated...
membranes with different contact angles. In our model, we assume that the contact angle affects the radius expansion rate

\[ \nu \propto \frac{1}{\cos \alpha} \quad (\text{Eqs. (2) and (3)),} \]

with varying pore sizes. In our model, we assume that the pore size affects the radius expansion rate

\[ \nu \propto \frac{1}{D} \]

displacement (i.e., increasing pressure on the device) did not cause a significant difference between the overall current levels (Fig. 3d, left and middle panels). A control experiment showed that the gold nanoparticles assembled in curli were critical for making the colonies conductive, as pressing together two colonies containing no gold nanoparticles generated no detectable current (Fig. 3c, black lines; Fig. 3d, right panel).

In contrast, the domes containing gold nanoparticles exhibited differential pressure responses. To control the dome shape, we grew the bacteria carrying the curli-pattern circuit on PC membranes with different pore sizes, before incubating with gold nanoparticles. The resulting colonies had different radii but were approximately the same height (Supplementary Fig. 2b). The height was sufficiently small such that two colonies placed opposite each other in our device were not in contact without being pressed. Thus, in the off-state (without externally applied pressure), the electrical current through the device was near zero. When the two colonies were pressed into contact, the resulting current increased with increasing applied pressure (Fig. 3e). For the colonies without a dome structure, the maximal responses to the first two presses were similar (Fig. 3d, right panel). That is, these colonies did not exhibit differential pressure responses. In contrast, for the colonies with dome structure, the maximal response to the second press was twice as large as that to the first press,

Figure 2 Bacterial growth and pattern formation on permeable membranes. (a) The bacterial colonies were grown on permeable membranes. We loaded 0.3% molten agar in 2×YT with IPTG and appropriate antibiotics in a multiwell chambered coverslip. After the agar solidified, we placed a permeable membrane on top of the culture well and printed bacteria onto the membrane surface. The diagram is not to scale. (b) Experimentally generated dome structures on membranes with different pore sizes. Each column represents the heat map of mCherry fluorescence patterns measured by a confocal microscope after 32 h incubation in both vertical (y axis) and radial (x axis) directions. The pore size varied from 0.03 to 0.4 μm, as indicated. The contact angles of these membranes varied slightly (from left to right: 64.0°, 59.0°, 58.5°, 57.7°, 55.3°). (c) Simulated dome structures on membranes with varying pore sizes. In our model, we assume that the pore size affects the radius expansion rate ν and the nutrient influx rate α1 (Eqs. (2) and (3)), respectively. Each column represents the heat map of simulated mCherry fluorescence patterns for the varying pore sizes. (d) Experimentally generated dome structures on membranes with different contact angles. Each column represents the heat map of mCherry fluorescence patterns measured by a confocal microscope after 32 h incubation in both vertical (y axis) and radial (x axis) directions. From left to right, the membrane is PVDF (polyvinylidene difluoride), PC, MCE (mixed cellulose ester), NC (nitrocellulose). The pore size of each membrane is 0.45 μm. Contact angle of each membrane is 134.3°, 63.1°, 38.0°, 1.7°. The far left image is a colony directly grown on 0.3% 2×YT (pH = 6.5) agar. (e) Simulated dome structures on membranes with different contact angles. In our model, we assume that the contact angle affects the radius expansion rate ν according to Eq. (2). Each column represents the heat map of mCherry fluorescence using a simulation with different ν.

by a programmable syringe pump, pressing on the cylinder (Supplementary Fig. 4c).

The displacement of the actuator had different profiles, depending on how fast and how strong two colonies were pressed together or separated (Fig. 3a,b). We first tested the pressure response of colonies not containing the programmed structures. We used MG1655 ΔcsgA cells expressing the histidine-tagged CsgA under the induction of AHL21. When curli expression was fully induced, the assembled gold nanoparticles were distributed approximately uniformly in the colonies, forming a solid spherical cap (Supplementary Fig. 3c, third column). We controlled the radii and heights of different colonies by using membranes with different pore sizes, where a larger pore size generates colonies with larger radii and height. Because each colony’s height was >250 μm, the two opposing colonies were already in contact even without applied pressure (Fig. 3c, orange line), leading to a high base-level current. Although the current increased when the two colonies were pressed together, it did not significantly vary with different displacement profiles (Fig. 3c, magenta line). The lack of a strong differential response was more evident when we plotted the current response against the displacement distance. Increasing displacement (i.e., increasing pressure on the device) did not cause a significant difference between the overall current levels (Fig. 3d, left and middle panels). A control experiment showed that the gold nanoparticles assembled in curli were critical for making the colonies conductive, as pressing together two colonies containing no gold nanoparticles generated no detectable current (Fig. 3c, black lines; Fig. 3d, right panel).

In contrast, the domes containing gold nanoparticles exhibited differential pressure responses. To control the dome shape, we grew the bacteria carrying the curli-pattern circuit on PC membranes with different pore sizes, before incubating with gold nanoparticles. The resulting colonies had different radii but were approximately the same height (Supplementary Fig. 2b). The height was sufficiently small such that two colonies placed opposite each other in our device were not in contact without being pressed. Thus, in the off-state (without externally applied pressure), the electrical current through the device was near zero. When the two colonies were pressed into contact, the resulting current increased with increasing applied pressure (Fig. 3e). For the colonies without a dome structure, the maximal responses to the first two presses were similar (Fig. 3c), even though the second press was stronger than the first. That is, these colonies did not exhibit differential pressure responses. In contrast, for the colonies with dome structure, the maximal response to the second press was twice as large as that to the first press,
Figure 3 Patterned gold nanoparticles as a resettable pressure sensor. (a) Two opposing colonies were compressed with controlled distance. The distance indicates the displacement of the presser from its starting position. The presser starts to make contact with the device when the displacement is >1 mm. The vertical gray columns in a, c, and e indicate the contact time between pressing device and colony sensor. (b) Colony representation at the time points indicated in a, with the gold nanoparticles binding curli within the dome. (c) Displacement of the actuator over time. Magenta and orange lines indicate currents from colonies grown on membranes with pore sizes of 0.03 µm and 0.1 µm, respectively (solid yellow cap indicates colony with uniform nanoparticle distribution; arrows indicate electric current). The black line indicates response of colonies of pattern-forming bacteria grown on membrane with a pore size of 0.03 µm without assembling gold nanoparticles (red spherical cap). (d) Intensity of electric current as a function of the pressing distance for colonies not containing dome-structured gold nanoparticles. Shown are responses from a pair of colonies grown on a membrane with a pore size of 0.03 µm without assembling gold nanoparticles (left panel), 0.1 µm (middle panel), and 0.1 µm without assembly of gold nanoparticles (right panel). Light and dark lines indicate a varying pressing distance from 0–1.5 mm and 0–1.8 mm respectively. (e) The red, blue, and green solid lines indicate responses from colonies grown on membranes with pore sizes of 0.05 µm, 0.2 µm, and 0.4 µm, respectively. The dashed blue line represents a replicate experiment of the solid blue line by using a different electrochemical machine of the same model on a different day. (f) Intensity of electric current as a function of the pressing distance for colonies containing dome-structured gold nanoparticles. Shown are responses from a pair of colonies grown on a membrane with a pore size of 0.05 µm (left panel), 0.2 µm (middle panel), and 0.4 µm (right panel). The light and dark lines indicate a varying pressing distance from 0–1.5 mm and 0–1.8 mm, respectively. The green line indicates a varying pressing distance from 0–1.8 mm.

demonstrating differential pressure response (Fig. 3e). We speculate that with increasing pressure, the deformation of the dome structure would cause tighter packing of nanoparticles near the contact point. This in turn would increase the number of conducting pathways through the device, and as a consequence, an increased electrical current (Fig. 3e, red arrows).

We also found that devices with smaller domes (i.e., those with smaller radii of curvature) had a stronger pressure response than one containing larger domes (Fig. 3e). This response is likely due to two reasons. First, different domes would have similar electrical resistivity because the densities of gold particles would be similar among all domes. Thus, a smaller dome would have a smaller resistance than a larger dome. Second, given the same pressing distance, the strain is higher in a smaller dome compared with that in a larger dome at the same relative location within the colony. Therefore, there will be more particle-particle contact in smaller domes, which in turn would increase the conductivity. These interpretations are consistent with the results from finite element simulations of strain and pressure experience by two domes when pressed (Supplementary Fig. 5). Because these domes were elastic, the device could be pressed multiple times and show a re-settable and robust readout (Fig. 3e and Supplementary Figs. 6 and 7). When the pressing distance increased, there was a dramatic increase in current flowing through a device with two small domes (Fig. 3f). The differential pressure response was smaller for a device with two larger domes (Fig. 3f).

In contrast to solid spherical cap structures, the dome-shaped structures had strong differential responses to pressure (Fig. 3c,e). In a solid spherical cap structure with gold nanoparticles uniformly distributed, there were more conducting pathways for electrons (Fig. 3c, red arrows) than in a dome structure (Fig. 3e, red arrows). Thus, a change in the pressing distance between two domes would have little effect on colony resistance. Given the same change in the pressing distance, the number of conductive pathways will increase more dramatically with an increasing contact area in a dome structure.

Due to the viscoelastic behavior of the bacterial colonies, the distribution of gold nanoparticles embedded in the domes would likely respond with different conduction dynamics to different pressing profiles (Fig. 4a). Without pressure, the distribution of gold nanoparticles in each dome was similar (time point 1). When two domes were pressed together (time point 2), the contact region of the two opposing domes increased and the exerted pressure led to a densification of the local distribution of gold nanoparticles. If the displacement of the actuator was maintained, that is, the strain on the device was held constant (the flat region between points 2 and 3, Fig. 4a), then the density of the gold nanoparticles in the contact region should decrease as the stress in the visco-elastic matrix relaxes (Fig. 4b), and should lead to a concomitant decrease in conductivity. In other words, at constant strain we expect to see the current flow through the device to drop. Our experimental observations confirmed this prediction.
and green solid lines indicate current responses from colonies grown on strengths (Fig. 5b).

The ability of the sensors to transduce differential pressure inputs (Supplementary Video 1). As an illustration, we used a pressure sensor consisting of two fac-

Distances of gold nanoparticles corresponding to different pressure inputs. In 1) the pressure is zero; in 2) the pressure and its derivative are both positive; and in 3) the pressure is positive, but its derivative is zero. The three time points are labeled in a. (c) The response of the pressure sensor to changing pressure. The blue and green solid lines indicate current responses from colonies grown on membranes with pore sizes of 0.2 μm and 0.4 μm, respectively.

Figure 4 Patterned gold nanoparticles respond to pressure derivatives. (a) The pressing distance as a function of time, with the same device configuration as in Figure 3a. (b) Distributions of gold nanoparticles corresponding to different pressure inputs. In 1) the pressure is zero; in 2) the pressure and its derivative are both positive; and in 3) the pressure is positive, but its derivative is zero. The three time points are labeled in a. (c) The response of the pressure sensor to changing pressure. The blue and green solid lines indicate current responses from colonies grown on membranes with pore sizes of 0.2 μm and 0.4 μm, respectively.

(No...
the center of the colony was carefully adjusted manually. The two pressers were controlled with the same mechanical pump to synchronize their operation. The units of axes are of the same scale among panels i–iv. The units of x axes are all in µm and 0.05 µm, respectively, and used separately or in combination. The y axes are of the same scale among panels i–iv. The units of y axes are all in µA. To obtain reproducible results, all voltage providers used in the electronic circuits are of the same model (Keithley Series 6400 Picoammeters). The vertical alignment between the bottom of the presser and the center of the colony was carefully adjusted manually. The two pressers were controlled with the same mechanical pump to synchronize their operation.

Figure 5 Robust signal processing by the bacterial pressure sensors. (a) Control of an LED light using a bacterial pressure sensor in response to manual operation. The images indicate LED light intensities when the sensor was pressed to varying degrees or released (also see Supplementary Video 1). (b) Construction of a noise filter and a signal amplifier using bacterially assembled gold domes. As in Figure 3e, the input is the pressing distance as a function of time. Two sets of colonies were grown on the membranes with pore sizes of 0.2 µm and 0.05 µm, respectively, and used separately or in combination. The x axes are of the same scale among panels i–iv. The units of y axes are all in µA. To obtain reproducible results, all voltage providers used in the electronic circuits are of the same model (Keithley Series 6400 Picoammeters). The vertical alignment between the bottom of the presser and the center of the colony was carefully adjusted manually. The two pressers were controlled with the same mechanical pump to synchronize their operation.

tunability (varying height or width of the colony) primarily by controlling membrane properties. However, pattern formation can be further tuned by adjusting circuit parameters, such as the strength of positive feedback, the burden of circuit activation, or the strength of cell-cell communication\textsuperscript{23,26}. Alternative circuits can generate other patterns\textsuperscript{31,32,34} by one or multiple engineered populations, and the engineered curli can be replaced by other effector molecules to assemble soft materials, such as self-organized hydrogel formation\textsuperscript{50}. Other organisms, such as yeast, could allow further variations in pattern formation\textsuperscript{51}. Engineering at multiple time and length scales could enable the predicable assembly of materials for diverse applications in medicine\textsuperscript{52,53}, biotechnology\textsuperscript{54,55}, and environmental cleanup\textsuperscript{56,57}. The ability to generate programmable 3D patterns may also facilitate the study of the design principles of natural 3D patterning processes, such as skeletal patterns in limb\textsuperscript{58}, tooth\textsuperscript{59,60,61}, and biofilms\textsuperscript{61,62}.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We thank R. Tsoi, C. Zhang, Z. Dai for discussions and comments; Y. Gao for assistance with confocal microscopy; Duke Light Microscopy Core Facility (LMCF) for access to confocal microscopes and imaging software; M. Plue for assistance with the TEM and SEM; Duke Shared Materials Instrumentation Facility (SMIF) for access to TEM and SEM. This study was partially supported by the Office of Naval Research (N00014-12-1-0631), National Science Foundation (L.Y.: MCB-1412459; M.D.R.: DMS-1614838), Army Research Office (L.Y., #W911NF-14-1-0490), National Institutes of Health (L.Y.: 1R01-GM098642; K99CA207872-01), Swiss National Science Foundation (M.D.R.: P300P2_154583), a David and Lucile Packard Fellowship (L.Y.).

AUTHOR CONTRIBUTIONS

L.Y. and Y.C. conceived the project. Y.C. generated and analyzed all the experimental data. Y.C. developed MATLAB codes for image analysis. Y.C. and Y.F. designed and carried out the electrochemical pressure-sensing experiments. Y.C. conducted parameter fittings in all simulations and generated the final simulation results. C.C. developed the finite element simulations for strain analysis. K.Z. assisted with immunolabeling and TEM imaging. K.M. assisted with TEM imaging. Y.C., S.Z., and L.Y. wrote the manuscript, with input from Y.F., M.D.R., K.Z., G.H., and K.M.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Plasmids, cell strains, and growth media. The curli-pattern circuit consists of two plasmids: pET15bLCFPT7 and pTuLysCsgAHis2CMR2, as described previously21. The csgAHis gene23 was inserted downstream of the T7 lysozyme gene to form the curli-pattern circuit. As a control, we used pZA-Cmr-rr12y-pLuxR-csgA ompR234 for induced expression of curli protein. Unless noted otherwise, MG1655 PRO ΔcsgA ompR234 cells carrying the curli-pattern circuit or the control plasmid was used for the printing experiments. For initial overnight culture, LB medium was used; for liquid culture experiments, M63 minimum medium supplemented with 0.2% w/v glucose and 1 mM MgSO4 was used; for growth on membrane experiments, 2×YT medium was used61. All media were adjusted with 1.0 M KOH (Sigma) solution to pH = 6.5 by VWR Symphony SB70P PH meter.

Measurement of cell density in liquid culture. Cell densities of liquid cultures were quantified using optical density (OD) measured at 600 nm absorbance using a Perkin-Elmer VICTOR3 plate reader.

Transmission electron microscopy (TEM). 10 µl fixed sample was deposed onto a 200-mesh formvar/carbon-coated nickel TEM grid (Electron Microscopy Sciences) for 2 min, then stained with 2% uranyl acetate (Electron Microscopy Sciences) for 30 s. TEM images were obtained on FEI Tecnai G2 Twin transmission electron microscope at 80 kV accelerating voltage. All these parameters were kept the same between experiments.

Scanning electron microscopy (SEM). A 1 cm x 1 cm silicon wafer was immersed in 1 ml sample solution overnight. After the fixation and dehydration process, wafer with samples were imaged under FEI XL30 SEM-FEG scanning electron microscope with 10 kV accelerating voltage. Images were obtained at ultra-high resolution, using the secondary electron imaging mode. All these parameters were kept the same between experiments.

Confocal microscopy. Samples were imaged with a Zeiss 780 confocal upright fixed-stage confocal microscope using a 10x/0.45 Dry Zeiss Plan-Apochromat 1063-139 WD 2.0 mm objective. For mCherry observation, the samples were excited at 561 nm, the emission was detected through 576 nm and 696 nm bandpass filters. All these imaging parameters were kept the same between experiments.

Electrochemical measurement. Currents though the device were measured with a Bio Logic SP-200 electrochemistry workstation, at 1 V applied voltage.

Inkjet printing64. We used the Epson Stylus Photo R280 Ultra Hi-Definition Photo Printer (C11C691201) for printing experiments for three reasons64. First, this printer contains a CD tray, which provides the capability of printing on a solid flat surface. After the culture well was loaded on the CD tray, the printing template could be designed to match the corresponding position of the culture well. Second, the inkjet is piezo-activated, which will not affect the cell viability. Third, the printer has a high resolution: 5,760 x 1,440 pixels at the maximal dots per inch (dpi), which enables precise control of initial seeding positions of bacteria.

To facilitate the manipulation and sterilization, the outer shell of the printer was disassembled and removed. We then used PrintPayLess six packs Empty Refillable Ink Cartridges instead of the original ink cartridges.

Print heads were cleaned thoroughly before and after each experiment. First, the printer head box was repositioned to the middle of the printer tray and absorbent paper towels were placed under the printer head to collect the liquid flushing through the printer heads. Second, the printer heads were flushed with 75% ethanol once, followed with washing with deionized water three times gently using a syringe. The absorbent paper towels were removed and the printer head box was then placed back in its original spot.

To prepare 0.3% agar for printing, we mixed 0.15 g of agar (214530 Difco Agar, Granulated) in 50 ml of 2×YT medium, and microwaved the mixture until it was homogenous with no aggregates. We then cooled the agar below 50 °C at room temperature, and supplemented it with 50 µg/mL kanamycin, 50 µg/mL spectamycin, 75 µg/mL carbencillin, 50 µg/mL chloramphenicol, and 1000 µM IPTG and 100 nM acyl-homoserine lactone (AHL). We next pipetted 170 µL of the agar into each culture well, and let it solidify at room temperature. An overnight culture of MG1655 ΔcsgA cells carrying the full circuit was diluted to 0.2 absorbance (measured by Victor 3 plate reader) and then diluted another 50-fold into fresh LB broth. The diluted culture was transferred into an ink cartridge empty of toner using a sterile syringe. The other five cartridges were filled with deionized water sterilized using a 0.2 µm filter (VWR Syringe Filters, # 28145-477).

Before printing, a porous membrane (Whatman, plc) was placed on top of the solidified agar. Printing templates were designed in software GIMP using 1-pixel diameter spot. Each template was exported to an Epson CD printer program to direct printing of bacteria onto the membrane surface. After printing, the whole device was incubated under 30 °C for 32 h.

NINTA-AuNP labeling. The protocol is adapted from earlier work21:

1. 20 µL of bacterial culture was placed on parafilm with TEM grid floating on top. The coated side of the TEM grid was in contact with the culture for 2 min.
2. The TEM was washed by 20 µL of 1× phosphate-buffered saline (PBS) 5 times (1 min each time).
3. The TEM grid was placed on top of 20 µL of selective binding buffer (1 × PBS with 0.487 M NaCl, 80 mM imidazole, and 0.2% Tween20) 3 times (1 min each time).
4. The TEM grid was placed on top of 50 µL of selective binding buffer with 10 nM 5 nm NINTA-AuNP particles (Nanoprobes) for 90 min.
5. The TEM grid was washed 5 times (1 min each time) with 20 µL of selective binding buffer, and 3 times (1 min each time) with 20 µL of 1 × PBS.
6. Before imaging under TEM, the grid was stained with filtered 2% uranyl acetate for 30 s.

Immunolabeling nanoparticles to the colonies (Supplementary Fig. 3a).

1. After peeling the membrane from the agar, a membrane was washed twice by floating on top of 1× PBS twice, each wash lasted for 5 min.
2. The membrane was placed to float on top of 8% formaldehyde for 24 h to fix the colony. After the fixation, membrane was taken out and air-dried for 5 min.
3. The membrane was washed with PBS twice by floating on top of 1× PBS, each wash lasted for 5 min.
4. After wash, membrane was placed on top of blocking buffer (6% BSA (RIA grade, Sigma Cat. no. A-7888) in 1 × PBS) for 1 h at room temperature.
5. The membrane was incubated in the primary antibody (mouse anti-6× His tag antibody conjugated with biotin, Thermo Fisher Scientific, # MA1-21315-BTIN) diluted 1,000-fold in blocking buffer overnight at 4 °C.
6. Followed with three times 1× PBS wash (5 min each wash), the membrane was incubated in the secondary antibody (goat anti-Mouse IgG conjugated with 10 nm gold particles, Life Science Technologies, LLC, # G7652) diluted tenfold in blocking buffer for 2 h under room temperature (note: secondary antibody in Supplementary Figure 1h is Streptavidin-655 Qdots, Life Science Technologies, LLC, # Q10121MP).
7. The membrane was washed in 1× PBS 3 times (5 min each wash).

In our experiments, gold nanoparticles were added to saturation, such that the resulting structures will be determined by the engineered dome patterns (as illustrated in Supplementary Fig. 3b). In particular, we chose the concentration such that: 1) the concentration is high enough to saturate the gold particles binding reaction; 2) the concentration cannot be too high to cause self-aggregation of the gold nanoparticles. In particular, when the concentration such that: 1) the concentration is high enough to saturate the gold particles binding reaction; 2) the concentration cannot be too high to cause self-aggregation of the gold nanoparticles. After multiple tests, we found that tenfold dilution of the original gold nanoparticle conjugates to be the optimal dilution.

Finite element simulations (Supplementary Fig. 5). To provide insights into how domes with different radii responded differently to the same pressing, we performed finite element simulations for the compression process. All these simulations were carried out using the commercial finite element
The height of all dome structures is 200 μm. The radii of small and large domes are 300 and 420 μm, respectively. The thickness of the mixture layer \((t)\) from cell and gold nanoparticles is assumed to be 1/5 of the dome height (Supplementary Fig. 5a, left). Based on the mechanical properties of biofilm and polymers reported in the literature, we modeled the elastic colony dome as an incompressible neo-Hookean material with Young's modulus of \(E_1 = 100\) MPa. The mixture layer of cells and gold nanoparticle with same model was assumed to a larger modulus of \(E_2 = 200\) MPa. Eight-node linear brick, hybrid elements, with reduced integration (C3D8RH) were used for all the simulations and a mesh sensitivity study was carried out to ensure the accuracy of the results (Supplementary Fig. 5a, right). A total of 228,656 and 111,392 C3D8RH elements were used for the larger and smaller domes, respectively. A perfect bonding between the two different layers was assumed. The bottom surface of the lower dome was completely fixed, and a displacement load of 50 μm was incrementally applied to the top surface of the upper dome. The contact model between two domes was assumed to be general smooth contacts without friction. Quasi-static nonlinear simulations were performed using ABAQUS/Standard.

For the pressure sensor consisting of a pair of domes, the total electrical resistance includes two parts: one is the materials resistance from the two colonies with dome structures, the other is the contact resistance between the contacting interface of the colony domes, which is the dominated factor that affects the conductivity of the devices.

For rough surfaces with microscale and nanoscale structures, the electrical contact resistance between two conductors is controlled by contact pressure and interface smoothness. Regardless of the interface smoothness, the resistance between the pair of domes should decrease with the contact pressure. Our simulations show that the smaller dome demonstrates a larger (14.5% more) average contact pressure at the contact surface area of the domes (Supplementary Fig. 5d), resulting in a smaller resistance and thus a larger current flow. Our simulations show that this conclusion is still maintained for a wide range of variations in the modulus ratio of \(E_2/E_1\) of the materials (Supplementary Fig. 5e) and the mixed layer's thickness (Supplementary Fig. 5f).

When compressed at the same distance (50 μm), the smaller domes experience much higher strains than do the larger domes (Supplementary Fig. 5b,c). The average strain along the middle interface of the conducting mixture layer in the smaller domes is ~23.8% larger than that in the larger colony domes. Therefore, more particle-particle contacts can be expected in smaller domes, which would consequently reduce the resistance and in turn increase the total conductivity.

**Model development.** Colony growth dynamics. To better understand and predict the experimental outcomes, we develop a phenomenological model to describe colony growth and gene expression. In particular, by assuming the separation of time scales, we decouple the colony growth from the gene circuit dynamics by first modeling growth in the absence of the circuit and then considering the gene expression based on the final shape of the colony.

Because of its radial symmetry when growing on top of a flat membrane (Fig. 2a), we characterize the colony by two variables:

1. The radial extension \(R(t)\) on the membrane at time \(t\)
2. The height function \(h(r, t)\), which determines the height of the columns of cells situated at a distance \(r\) from the center of the colony.

Colony growth is regulated by the amount of nutrient in the colony, \(N_{\alpha}(t)\), which in turn depends on the amount of nutrient in the agar, \(N_{\alpha}(t)\).

Our model consists of the following differential equations:

\[
\begin{align*}
\dot{R} &= v \frac{n_{\alpha}}{n_{\alpha} + K_p} \\
\dot{h}(r, t) &= \gamma \frac{n_{\alpha}}{n_{\alpha} + K_p} h(r, t) \left( \frac{Q_m}{Q_m + h(r, t)^m} \right) \quad 0 \leq r \leq R \\
\dot{n}_{\alpha} &= -\delta_1 R^2 (n_{\alpha} - n_c) \\
\dot{N}_{t} &= \alpha_2 n_{\alpha}^2 \left( n_{\alpha} - n_c \right) - \beta_m \frac{n_{\alpha}}{n_{\alpha} + K_p} V_c - \beta_p \frac{n_{\alpha}}{n_{\alpha} + K_p} V_p
\end{align*}
\]

where,

\[
\frac{n_{\alpha}(t)}{V_{\alpha}} \ 	ext{is the nutrient concentration in the agar at time } t \text{ and } V_{\alpha} \text{ is the volume of the agar;}
\]

\[
\frac{N_{\alpha}(t)}{V_{\alpha}} = \int_{r<R(t)} h(r) dr \ 	ext{is the volume of the colony at time } t;
\]

\[
\frac{n_{\alpha}(t)}{V_{\alpha}} = \frac{N_{\alpha}(t)}{V_{\alpha}} \ 	ext{is the nutrient concentration in the colony at time } t;
\]

\[
V_p = \int_{r>R} h(r) \frac{Q_m}{Q_m + h(r)^m} dr \ 	ext{is the total volume of cells that undergo proliferation;}
\]

other constants in Eq (1) are explained in Supplementary Table 1.

In deriving the above equations, we made the following assumptions:

1. The colony expands radially at a speed that depends on the nutrient concentration \(n_{\alpha}\), analogous to Monod kinetics (see ref. 26 for details). With saturating nutrients, the colony exhibits traveling wave solutions with an asymptotic speed \(v\). Because cells consume nutrients, depletion of nutrient leads to decrease in the wave speed as modeled by means of the \(\frac{n_{\alpha}}{n_{\alpha} + K_p}\).

2. At each position \(r\) where the colony touches the membrane \(0 \leq r \leq R\), the colony has a vertical growth rate \(h(r, t)\) that increases with the availability of nutrient \(\frac{n_{\alpha}}{n_{\alpha} + K_p}\) and decreases with the height \(\frac{Q^m}{Q^m + h(r)^m}\).

3. We assume fast diffusion of nutrient in both agar and colony, leading to a homogeneous distribution in each compartment. Nutrient transfer from agar to colony takes place across the membrane. The rate of exchange is proportional to the area of contact between colony and membrane and the concentration gradient between the two compartments. The nutrient exchange accounts for the third equation and the first term in the fourth equation in (1).

4. In the colony, nutrient is depleted by the cells. We assume all cells to have a baseline metabolic resorption rate, captured by the second term in the fourth equation in (1). The last term in the equation accounts for depletion by cells undergoing proliferation.

In the experiments, the membrane pore size \(\rho\) and hydrophobicity (as measured by the contact angle \(\theta\)) were varied as control parameters. To account for the impact of these parameters on the model, we assume that the radius expansion rate \(v\) is a function of the pore size \(\rho\) and the membrane contact angle \(\theta\), and that the fitting constant for nutrient loss by transport \(\alpha_2\) is a function of \(\rho\). More precisely, we use the following empirical equations:

\[
v = \left( \frac{20}{\left(1 + \frac{\theta}{40}\right)^{10}} \right) (v_1 \rho^2 + v_2)
\]

where \(v_1 = 0.25\ \mu m^{-1}\ hr^{-1}, v_2 = 0.95 \mu m\ hr^{-1}\) and

\[
\alpha_1 = v_3 \rho^2
\]

where \(v_3 = 0.01\ \mu m^{-4}\).

The contact angle made by water droplets and the surface provides a measure of hydrophobicity of the surface. Because the bacterial colony is mostly made of water, this will also provide us with a sense of how attracted the colony is to the surface. Water droplet equilibration is driven by surface energy, meaning that the contact angle may be used to determine the final shape of the droplet. On the other hand, colony growth is driven by interior mechanical forces (surface tension is negligible), meaning that the contact angle cannot be used to determine the final shape of the colony. Most importantly, the
surface interaction with the growing colony will affect the ratio between vertical and horizontal growth rates. The contact angle of the colony is an emerging, rather than driving factor in colony growth. Due to this observation along with the simplifications in our model, we do not expect the model to empirically recover the less important contact angle, but do expect the model to recover the more important ratio of colony height-to-width.

Before simulating the colony growth, we rescale the variables as

\[
\tilde{r} = \frac{n_r}{K_p}, \quad \tilde{n}_a = \frac{n_a}{K_p}, \quad \tilde{h} = \frac{h}{Q}, \quad \tilde{t} = ty
\]

The model equations can be rewritten using the parameter groups \(G_i\) (see Supplementary Table 2),

\[
\begin{aligned}
\dot{R} &= G_i \frac{\tilde{n}_a}{\tilde{n}_c + 1} \\
\dot{h}(r,t) &= \frac{\tilde{n}_a - \tilde{h}(r,t)}{\tilde{n}_c + 1} + \dot{h}(r,t) + R(n) \\
\dot{\tilde{n}_c} &= -G_2 R^2 (\tilde{n}_a - \tilde{n}_c) \\
\dot{\tilde{n}_a} &= G_3 R^2 (\tilde{n}_a - \tilde{n}_c) - G_4 \frac{\tilde{n}_a}{\tilde{n}_c + G_3} V_c - G_6 \frac{\tilde{n}_a}{\tilde{n}_c + 1} V_p
\end{aligned}
\]

Because systems (1) and (4) are infinite dimensional (the equation for \(h\) is defined for each \(r < R\)), we discretize the radial dimension of the colony into 100 equally spaced elements of size 50. We then solve the resulting system of ODEs using a stiff differential equation solver (ode23s) in MATLAB. The initial conditions are specified as: \(R(0) = 0.1, h(r,0) = 0.1 (0 \leq r < R(0)); \tilde{n}_c(0) = 50; \tilde{n}_a(0) = 0\). The computational domain only provides the computational range for running the simulation. Consequently, we choose a computational domain large enough to avoid pattern interference with the boundary and to enforce no-flux boundary conditions.

Expression profiles. Based on the model of the colony growth dynamics developed in the previous section, we can now quantify the final gene expression profiles. To this end, we use the above model to compute the colony shape in the quasi-stationary state where nutrient has been depleted. We then compute the corresponding steady-state profiles of T7RNAP (denoted as \(T\)) and T7 lysozyme (denoted as \(L\)), the T7-lysozyme complex (denoted as \(P\)), and AHL (denoted as \(A\)). The equilibrium equations for gene expression are given by the following nonlinear system (ref. 26 and Supplementary Table 2 for details),

\[
\begin{aligned}
\dot{A} &= 0 = -G_7 \int_{1}^{T} \frac{1}{1 + T + 1 + P} \varphi(r,K) dV - G_8 A \\
\dot{L} &= 0 = -G_3 L + G_10 \theta(C) T \frac{A^M}{1 + T + 1 + P} \varphi(r,K) \\
\dot{T} &= 0 = -G_11 T + G_12 \theta(C) T \frac{A^M}{1 + T + 1 + P} \varphi(r,K) \\
P &= \frac{G_3}{G_14} TL
\end{aligned}
\]

where the function \(\varphi\) represents the gene expression capacity,

\[
\varphi(r,C) = \begin{cases} 
\frac{K^n}{K^n + (R - r)^n}, & r \leq R \\
1, & r > R
\end{cases}
\]

With \(R\) the colony radius, see (1) and (4). In (6) and (7), \(\theta\) is the Heaviside function, \(\theta(x) = 1\) if \(x > 0\) and \(\theta(x) = 0\) otherwise. \(C\) is the cell density, when cell density reach the carrying capacity, \(C = 1\).

Ignoring \(A\) for a moment, the Eqs. (6) - (8) form a cubic polynomial system in \(T\) and \(L\). One can determine the solution of this system analytically and find that there is one zero root. The remaining roots are either complex, or one is positive definite and the other negative definite. We assume that the steady state will obtain the positive root if it exists, and the zero root otherwise.

At this point, given a value for \(A\), we can compute the steady state profile for \(T\) and \(L\), however, Eq. (5) will not be satisfied. To find a simultaneous solution we performed the following iterative procedure:

1. Make an initial guess for \(A = A(0)\).
2. Predict \(L(0)\) and \(T(0)\) based on \(A(0)\) by solving Eqs. (6) - (8).
3. Update the prediction for \(A(0)\) by solving Eq. (5), to determine \(A(1)\).
4. Repeat steps (2) and (3) until the solution has converged.

In practice, we found convergence after as few as three steps of the iterative procedure with the parameters listed in Supplementary Table 2. Finally, we note that the growth model is built on the assumption of an incompressible bacteria colony with a sharp interface. In reality, the proliferating colony front is less dense than the core—because of this difference, we expect to see differences in profiles near the colony boundary.

Statistical analysis. Statistical analyses were performed with MATLAB (R2015b). Data are presented as mean \pm s.d. with sample numbers \(n\) noted in the figure legends. For detailed information, please also refer to the Life Sciences Reporting Summary.

Data availability. The sequence data of plasmid pET15bLCFPT7 are available on Addgene, ID53545; the sequence data of plasmid pTuLys2CMR2 are available on Addgene, ID53544.
**Experimental design**

| Sample size | Sample sizes in terms of number of replicates are described along with Figures. No statistical methods were used to determine the sample sizes |
|-------------|---------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | No data were excluded from the analyses |
| Replication | Data are repeatable on different date, different machines (machines have the same model). For example, as shown in Figure 3e, the dash blue line is reproduced curve compared to solid blue line. |
| Randomization | The initial starting cell culture are all come from the same -80C bacterial stock tube. A tip was used each time to scoop few cells from the tube, the scooping locations are all random. |
| Blinding | Two researchers were present to collect the electrochemical data, one researcher do the testing without knowing the exact parameters, then did the analysis. |

**Statistical parameters**

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- **Confirmed**
  - The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
  - A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
  - A statement indicating how many times each experiment was replicated
  - The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
  - A description of any assumptions or corrections, such as an adjustment for multiple comparisons
  - The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
  - A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
  - Clearly defined error bars

See the web collection on [statistics for biologists](#) for further resources and guidance.
### Software

**Policy information about availability of computer code**

7. Software

Describe the software used to analyze the data in this study. 

**MATLAB R2015b**

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

### Materials and reagents

**Policy information about availability of materials**

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

**no restrictions**

**Antibodies**

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

**mouse anti-6× His tag antibody conjugated with biotin, Thermo Fisher Scientific, # MA1-21315-BTIN; goat anti-Mouse IgG conjugated with 10nm gold particles, Life Science Technologies, LLC, # G7652; Streptavidin-655Qdots, Life Science Technologies, LLC, # Q10121MP**

**Eukaryotic cell lines**

a. State the source of each eukaryotic cell line used.

b. Describe the method of cell line authentication used.

c. Report whether the cell lines were tested for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by iCLAC, provide a scientific rationale for their use.

**N/A**

### Animals and human research participants

**Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines**

**Description of research animals**

Provide details on animals and/or animal-derived materials used in the study.

**N/A**

**Policy information about studies involving human research participants**

**Description of human research participants**

Describe the covariate-relevant population characteristics of the human research participants.

**N/A**