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Detailed Terms
Stretchable living materials and devices with hydrogel–elastomer hybrids hosting programmed cells

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Living systems, such as bacteria, yeasts, and mammalian cells, can be genetically programmed with synthetic circuits that execute sensing, computing, memory, and response functions. Integrating these functional living components into materials and devices will provide powerful tools for scientific research and enable new technological applications. However, it has been a grand challenge to maintain the viability, functionality, and safety of living components in freestanding materials and devices, which frequently undergo deformations during applications. Here, we report the design of a set of living materials and devices based on stretchable, robust, and biocompatible hydrogel–elastomer hybrids that host various types of genetically engineered bacterial cells. The hydrogel provides sustainable supplies of water and nutrients, and the elastomer is air-permeable, maintaining long-term viability and functionality of the encapsulated cells. Communication between different bacterial strains and with the environment is achieved via diffusion of molecules in the hydrogel. The high stretchability and robustness of the hydrogel–elastomer hybrids prevent leakage of cells from the living materials and devices, even under large deformations. We show functions and applications of stretchable living sensors that are responsive to multiple chemicals in a variety of forms factors, including skin patches and gloves-based sensors. We further develop a quantitative model that couples transportation of signaling molecules and cellular response to aid the design of future living materials and devices.

Significance

The integration of genetically programmed cells into materials and devices will enable the power of biology to be harnessed for a wide range of scientific research and technological applications. Here, we use stretchable, robust, and biocompatible hydrogel–elastomer hybrids to host genetically programmed bacteria, thus creating a set of stretchable and wearable living materials and devices that possess unprecedented functions and capabilities. A quantitative yet generic model is further developed to account for the coupled physical and biochemical processes in living materials and devices. This simple strategy for designing living materials and devices not only provides tools for research in synthetic biology but also, enables applications, such as living sensors, interactive genetic circuits, and living wearable devices.


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hybrids that host various types of genetically engineered bacterial cells. We show that our hydrogels can sustainably provide water and nutrients to the cells, whereas our elastomers ensure sufficient air permeability to maintain viability and functionality of the bacteria. Communication between different types of genetically engineered cells and with the environment is achieved via transportation of signaling molecules in hydrogels. The high stretchability and robustness of the hydrogel–elastomer hybrids prevent leakage of cells from the living materials and devices under repeated deformations. We show applications uniquely enabled by our living materials and devices, including stretchable living sensors responsive to multiple chemicals, interactive genetic circuits, a living patch that senses chemicals on the skin, and a glove with living chemical detectors integrated at the fingertips. A quantitative model that couples transportation of signaling molecules and responses of cells is further developed to help the design of future living materials and devices.

Results

Design of Living Materials and Devices. We propose that encapsulating genetically engineered cells in biocompatible, stretchable, and robust hydrogel–elastomer hybrid matrices represents a general strategy for the design of living materials and devices with powerful properties and functions. The design of a generic structure for the living materials and devices is illustrated in Fig. L4. In brief, layers of robust and biocompatible hydrogel and elastomer were assembled and bonded into a hybrid structure (15). Patterned cavities of different shapes and sizes were introduced on the hydrogel–elastomer interfaces to host living cells in subsequent steps. The hydrogel–elastomer hybrid was then immersed in culture media for 12 h, so that the hydrogel can be infiltrated with nutrients. Thereafter, genetically engineered bacteria suspended in media were infused into the patterned cavities through the hydrogel, and the injection points were then sealed with drops of fast-curable pregel solution (Fig. S1). Because the hydrogel was infiltrated with media and the elastomer is air-permeable, hydrogel–elastomer hybrids with proper dimensions can provide sustained supplies of water, nutrient, and oxygen (if needed) to the cells. By tuning the dimensions of hydrogel walls between different types of cells and between cells and external environments, we can control the transportation times of signaling molecules for cell communication. Furthermore, the high mechanical robustness of the hydrogel, elastomer, and their interface confers structural integrity to the matrix even under large deformations, thus preventing cell escape in dynamic environments.

In this study, we chose polyacrylamide (PAAm)-alginat hydrogel (15, 17) and polydimethylsiloxane (Sylgard 184; Dow Corning) or Ecoflex (Smooth-On) silicone elastomer to constitute the robust hydrogel and elastomer, respectively. The biocompatibility of these materials has been extensively validated in various biomedical applications (20, 21). The sufficient gas permeation of the elastomer enables oxygen supply for the bacteria (22–24). If a higher level of oxygen is required, one may choose elastomers with higher permeability, such as Silbione (25), or microporous elastomers (23). In the hydrogel, the covalently cross-linked PAAM network is highly stretchable, and the reversibly cross-linked alginic network dissipates mechanical energy under deformation, leading to tough and stretchable hydrogels (14, 17, 26). More robust devices can be fabricated by using fiber-reinforced tough hydrogel (27). Robust bonding between the hydrogel and elastomer can be achieved by covalently anchoring the PAAm network on the elastomeric substrate (27). The Escherichia coli strain was engineered to produce the chemical inducer, IPTG, in response to a chemical signal. The cells were kept in the device placed in a humid chamber to maintain above 90% over 3 d without addition of growth media or immersed the living materials in subsequent steps. The hydrogel, elastomer, and their interface confers structural integrity to the matrix even under large deformations, thus preventing cell escape in dynamic environments.

To test whether bacteria could escape from the living devices, we placed the hydrogel–elastomer matrices containing RhamRVC/GFP cells (Fig. L4) in a humid chamber (relative humidity > 90%) without addition of growth media or immersed the living materials in the growth media at room temperature (25 °C) for 3 d. We also directly cultured the cells in growth media as a control. Thereafter, we performed flow cytometry analysis for bacteria retrieved from the living device to test the cell viability. As shown in Fig. 1C and Fig. S2, the viability of cells in the device placed in a humid chamber maintains above 90% over 3 d without addition of media to the device. This viability is similar to that of cells in the device immersed in media or cells directly cultured in media at room temperature over 3 d.

To test whether bacteria could escape from the living devices, we deformed the hydrogel–elastomer hybrids containing RhamRVC/GFP bacteria in different modes (i.e., stretching and twisting) as illustrated in Fig. 1B, and then immersed the device in media for a 24-h period. As shown in Fig. 1B and Fig. S3, the living device made of Ecoflex and tough hydrogel sustained a uniaxial stretch over 1.8 times its original length and a twist over 180° while maintaining its structural integrity. Furthermore, after immersing the device in media for 6, 12, 20, and 24 h, we collected the media surrounding the device and measured the cell...
population in the media over time via OD_{600} by UV spectroscopy (Fig. 1D); 200 μL media were streaked on agar plates after 24 h to check for cell escape and growth (Fig. 1D, Insets). Fig. 1D shows that bacteria did not escape the hydrogel–elastomer hybrid even under repeated mechanical loads (500 cycles). As controls, we intentionally created defective devices (with weak hydrogel–elastomer bonding) and observed significant escape and overgrowth of bacteria after immersing the samples in media (yellow curve in Fig. 1D). Because agar hydrogels have been widely used for cell encapsulation, we fabricated an agar-based control device that encapsulated RhamRCV/GFP bacteria with the same dimensions as the hydrogel–elastomer hybrid. In Fig. S4, it can be seen that these agar devices underwent failures even under moderate deformation (e.g., a stretch of 1.1 or a twist of 60°). Moreover, cell leakage from the agar devices occurred regardless of the presence of any deformation, likely because of the large pore sizes and sol–gel transition of the agar gel, allowing for escape of encapsulated bacteria (Fig. S5). These results indicate that our hydrogel–elastomer hybrids can provide a biocompatible, stretchable, and robust host for genetically engineered bacteria.

**Stretchable Living Sensors for Chemical Sensing.** We next show functions and applications enabled by the living materials and devices. Fig. 2A illustrates a hydrogel–elastomer hybrid with four isolated chambers to host bacterial strains, including DAPGRCV/GFP, AHLRCV/GFP, IPTGRCV/GFP, and RhamRCV/GFP. Signaling molecules were diffused from the environment through the hydrogel window into cell chambers, where they were detected by the bacteria. (B) Genetic circuits were constructed in bacterial strains to detect cognate inducers (i.e., DAPG, AHL, IPTG, and Rham) and produce GFP. (C) Images of living devices after exposure to individual or multiple inputs. Cell chambers hosting bacteria with the cognate sensors showed green fluorescence, whereas the noncognate bacteria in chambers were not fluorescent. Scale bars are shown in images.

**(Fig. S7).** This orthogonality makes the hydrogel–elastomer hybrid with encapsulated bacteria into a living sensor that can simultaneously detect multiple chemicals in the environment (Fig. 2C). About 2 h are required for each strain to produce significant fluorescence. Parameters that affect response times for the living sensor are discussed with a quantitative model below.

**Interactive Genetic Circuits.** Next, we integrated cells containing different genetic circuits into a freestanding living device to study cellular signaling cascades. We designed two bacterial strains that can communicate via the diffusion of signaling molecules through the hydrogel, although both were separated by an elastomer barrier within discrete chambers of the device (Fig. 3A). Specifically, we used a transmitter strain (aTcRCV/AHL) that produces the quorum-sensing molecule AHL when induced by aTc and a receiver strain (AHLRCV/GFP) with AHL-inducible GFP genes (5). We triggered this device with aTc from the environment to induce the transmitter cells, which resulted in AHL production and stimulation of receiver cells to synthesize GFP (Fig. 3C). In Fig. 3B, we plot the normalized fluorescence of bacteria in different cell chambers (i.e., transmitter and receiver in Fig. 3A) as a function of time after aTc was added outside the device. Because there is no GFP gene in the transmitter cells (aTcRCV/AHL), their chambers showed no fluorescence over time.
(Fig. 3B). It took longer response time (~5 h) for the receiver cells in the middle chamber to exhibit significant fluorescence compared with the cells in simple living sensors (Fig. 2A). Two diffusion processes (i.e., aTc from the environment to the two side chambers and AHL from the two side chambers to the central chamber) and two induction processes (i.e., AHL production induced by aTc in transmitters and GFP expression induced by AHL in receivers) were involved in the current interactive genetic circuits. As a control, when the transmitters (aTcRCV/AHL) in the device were replaced by a cell strain containing aTc-inducible GFP (aTcRCV/GFP) that cannot communicate with AHLRCV/GFP, no fluorescence was observed in the receiver (AHLRCV/GFP) chamber (Fig. 3D). Overall, the integrated devices containing interactive genetic circuits provide a platform for the detection of various chemicals and the investigation of cellular interaction among physically isolated cell populations.

**Living Wearable Devices.** To further show practical applications of living materials and devices, we fabricated a living wearable patch that detects chemicals on the skin (Fig. 4 A–D). The sensing patch matrix consists of a bilayer hybrid structure of tough hydrogel and silicone elastomer. The wavy cell channels could cover a larger area of the skin with a limited amount of bacterial cells (Fig. 4A). The living patch can be fixed on the skin by clear Scotch tape, with the hydrogel exposed to the skin and the elastomer exposed to the air. The compliance and stickiness of the hydrogel promote conformal attachment of the living patch to human skin, whereas the silicone elastomer cover effectively prevents the dehydration of the sensor patch (Fig. S8) (15). As shown in Fig. 4 B–D, the inducer Rham was smeared on the skin of a forearm before we adhered the living patch. The channels with RhamRCV/GFP in the living patch became fluorescent within 4 h, whereas channels with AHLRCV/GFP did not show any difference. As controls, no fluorescence was observed in any channels in absence of any inducer on the skin (Fig. S9A), whereas all channels became fluorescent in presence of both AHL and Rham (Fig. S9B). Although the inducers are used as mock biomarkers here, more realistic chemical detections, such as components in human sweat or blood, may be pursued with living devices for scientific research and translational medicine in the future.

As another application, a glove with chemical detectors integrated at the fingertips was fabricated (Fig. 4E). The stretchable hydrogel and tough bonding between hydrogel and rubber allow for robust integration of living monitors on flexible gloves. To show the capability of this living glove, a glove-wearer held cotton balls that have absorbed inducers. Those chemicals from the cotton ball would diffuse through the hydrogel and induce fluorescence at two of three bacterial sensors that contained IPTGRCV/GFP, RhamRCV/GFP, and AHLRCV/GFP (Fig. 4 F–H). For example, gripping a wet cotton ball that contained IPTG and Rham resulted in fluorescence at two of three bacterial sensors that contained IPTGRCV/GFP (Fig. 4 G and H, *) and RhamRCV/GFP (Fig. 4 G and H, **) on the glove within 4 h. The middle sensor containing AHLRCV/GFP (Fig. 4 G and H, **) remained unaffected. The living patch and biosensing glove show the potential of living materials as low-cost and mechanically flexible platforms for healthcare and environmental monitoring. Looking forward, we envision the design of living devices that can be wearable, ingestible, or implantable for applications, such as water quality alert, disease diagnostics, and therapy.

**Discussion and Modeling**

**Model of Living Materials and Devices.** In this section, we developed a model that quantitatively accounted for the coupled physical transportation and biochemical responses underlying the living sensor (Fig. 2). The operation of the living sensor (Fig. 2A) relied on two processes: diffusion of signaling molecules from the environment through the hydrogel window into cell chambers and induction of the encapsulated cells by the signaling molecules (28). Given the geometry of the sensor (Fig. 5A), we could approximate the transportation of inducer in the hydrogel and the cell chamber to follow the 1D Fick’s law:

\[
\frac{dl}{dt} = D_g \frac{d^2 l}{dx^2} \quad \text{for} \quad 0 \leq x < L_g
\]

and

\[
\frac{dl}{dt} = D_L \frac{d^2 l}{dx^2} \quad \text{for} \quad L_g \leq x < L_g + L_c,
\]

where \(x\) is the coordinate of a point in the hydrogel window or the cell chamber; \(L_g\) and \(L_c\) are the thicknesses of the hydrogel and cell chamber, respectively; \(t\) is the current time; \(l\) is the inducer concentration in hydrogel or medium in the cell chamber; and \(D_g\) and \(D_L\) are the diffusion coefficients of the inducer in hydrogel and medium, respectively.

To prescribe boundary conditions for Eqs. 1 and 2, the inducer concentration at the boundary between the environment and the hydrogel window is taken to be a constant \(I_0\), the inducer concentration and inducer flux are taken to be continuous across the interface between the hydrogel and cell chamber, and the elastomer wall of the cell chamber is taken to be impermeable to the inducers. Because the diffusion process begins at \(t = 0\), the inducer concentration throughout the hydrogel window and cell
Fig. 5. Model for the diffusion–induction process in living materials and devices. (A) Schematic illustration of the diffusion of signaling molecules from the environment through the hydrogel to cell chambers in the living device. (B) Diagram of GFP expression after induction with a small molecule chemical. (C) Inducer concentration profile throughout the hydrogel window and cell chamber at different times. (D) Typical inducer concentration in the cell chamber as a function of time. (E) The normalized fluorescence of different cell strains as a function of time after addition of inducer (n = 3 repeats). Dots represent experimental data, and curve represents the model.

chapter is zero when \( t \leq 0 \) as the initial condition for Eqs. 1 and 2. Furthermore, the consumption of inducers by the bacterial cells was taken to be negligible. In these experiments, we set \( L_g = 5 \times 10^{-4} \) m, \( L_c = 2 \times 10^{-6} \) m, and \( I_0 = 1 \) mM for IPTG diffusion–induction. The diffusion coefficients of inducers are estimated to be \( D_g = 3 \times 10^{-10} \) m\(^2\)/s and \( D_c = 1 \times 10^{-10} \) m\(^2\)/s based on previous measurements (29).

The diffusion equations together with boundary and initial conditions were solved numerically. In Fig. 5C, we plot the inducer concentrations in the cell chamber at different times. It can be seen that the distribution of inducers in the cell chamber is more uniform than that in the hydrogel because of the higher inducer diffusion coefficient in media than that in hydrogel. We further define the typical inducer concentration \( I_c \) as the inducer concentration at the center of the cell chamber [i.e., \( I_c = I(1, x = L_g + L_c / 2) \)]. In Fig. 5D, we plot the typical inducer concentration in the cell chamber as a function of time.

To characterize the GFP expression of the bacterial cells in the living sensor, we adopt a model from Leveau and Lindow (30). The inducers can bind with repressors or activators in a bacterial cell and induce the transcription of promoters. The induced promoters initiate the synthesis of nonfluorescent GFP ("GFP"). Meanwhile, the "GFP" in the cell is consumed because of the maturation into the fluorescent GFP ("GFP"), cell division, and protein degradation. The converted "GFP" also undergoes consumption because of cell division and protein degradation. Eventually, the syntheses and consumptions of "GFP" and "GFP" reach steady states in the cell (Fig. 5B). Denoting the numbers of "GFP" and "GFP" in a cell as \( n \) and \( f \), respectively, their rates of variation can be approximated as

\[
\frac{dn}{dt} = P \cdot m \cdot n - \mu \cdot n - C_n
\]

and

\[
\frac{df}{dt} = m \cdot n \cdot f - C_f.
\]

In Eqs. 3 and 4, \( P \) is the promoter activity that expresses "GFP"; \( m \cdot n \) prescribes the maturation rate of "GFP" into "GFP", where \( m \) is the maturation constant; \( \mu \cdot n \) and \( m \cdot f \) prescribe the consumption rates of "GFP" and "GFP" caused by cell division, respectively, where \( \mu \) is the growth constant; and \( C_n \) and \( C_f \) are the degradation rates of "GFP" and "GFP", respectively. The promoter activity for transcription and translation induced by an inducer is approximated by a Hill equation (31) \( P = P_{max}I^n/(I^n + K^n) \), in which \( P_{max} \) is the maximum rate of "GFP" expression (i.e., maximum promoter activity), \( h \) is the Hill coefficient, and \( K \) is the half-maximal parameter (inducer concentration at which \( P \) equals \( 0.5 \cdot P_{max} \)). Because the inducer concentration in the cell chamber is relatively uniform (Fig. 5C), we take \( I \) in the promoter activity expression to be the typical concentration in the cell chamber [i.e., \( I = I_c \)]. Evidently, the connection between the transportation of inducers and biochemical responses of cells in the living sensor is through this Hill equation. Because the half-life of GFP in E. coli is over 24 h in absence of any proteolytic degradation, much longer than the typical responsive time of the living sensor, we assume \( C_n = C_f = 0 \) throughout this study. For the IPTG/GFP strain, we take \( P_{max} = 10^3 \) s\(^{-1} \), \( K = 0.3 \) mM, \( h = 2 \), \( m = 1.16 \times 10^{-2} \) s\(^{-1} \), and \( \mu = 1.20 \times 10^{-4} \) s\(^{-1} \) based on previously reported data on this system (30, 32).

In Fig. 5E, we plot the normalized fluorescence of cell in the device as a function of time after the inducers are added outside the living device. It takes around 2 h for different strains in the living sensor to show significant induced fluorescence (e.g., 0.5 of the maximal fluorescence). For the IPTG/GFP strain, the diffusion–induction–coupled model matches very well with experimental data (Fig. 5E).

Critical Timescales for Living Materials and Devices. From the above analysis, we know that the responsive time of a living material or device is determined by two critical timescales: the time for inducers to diffuse and accumulate around cells to the level that is sufficient for induction \( t_{diffuse} \) and the time to induce GFP expression and reach a steady-state \( t_{induce} \). To obtain analytical solutions for \( t_{diffuse} \), we develop a simple but relevant model as illustrated in Fig. S10A. The model is similar to the geometry of the living sensor (Fig. 5A) but assumes that the cells are embedded in a segment of hydrogel close to the elastomer wall. The inducer concentration in the environment is taken to be constant \( I_0 \), and the total thickness of the hydrogel is \( L \). By means of imaginary sources (33), the inducer concentration at location \( L \) (at the end of hydrogel) and time \( t \) can be expressed as

\[
I(L, t) = I_0 = 2erfc[(L - D_g t)/(2 \sqrt{D_g} t)] - erfc[L/(2 \sqrt{D_c} t)],
\]

where \( erfc(x) \) is the complementary error function. From Fig. S10B, it can be seen that the simplified model can consistently represent the typical concentration profile in the cell chamber of the living sensor. From promoter activity expression, we assume that, only when the inducer concentration at a point reaches the level of \( K \) (i.e., \( P = P_{max} / 2 \)), the inducer concentration is sufficient to induce the cells. Therefore, the critical diffusion time for cells with a typical distance \( L \) from the environment is

\[
t_{diffuse} = \left[ \frac{\Lambda(K/I_0)}{I_0} \right]^{-2} \times \frac{L^2}{D_g},
\]

where \( \Lambda(x) \) is the inverse of function of \( \Omega(x) = 2erfc(x/2) - erfc(x), L^2/D_g \) is the typical diffusion timescale, and the prefactor \( [\Lambda(K/I_0)]^{-2} \) accounts for the difference between \( I_0 \) and \( K \). We further fit the prefactor into a power law that approximately gives \( t_{diffuse} \approx 4/9 \times I_0(K - 1)^{0.56} \times L^2/D_g \) (Fig. S11).

We next evaluate the timescale to induce the cell in the device. When the inducer concentration around a cell reaches the level of \( K \) (i.e., \( P \) reaches the level of \( 0.5P_{max} \)), significant induction (e.g., expression of GFP) will occur in the cell. To solve Eqs. 3 and 4 analytically, we assume that the induction happens only after \( P \) reaches the level of \( 0.5P_{max} \) and \( P \) maintains at a constant level (between \( 0.5P_{max} \) and \( P_{max} \)) during the induction. Therefore, we can set \( n = f = 0 \) as the initial condition and \( P \) as a time-independent constant in Eqs. 3 and 4. Further setting \( C_n = C_f = 0 \), we can obtain analytical solutions \( n = P(1 - e^{-(m+\mu)P})/(m+\mu) \) for "GFP" and \( f = P(e^{-(m+\mu)P} - 1)/(m+\mu) - P(e^{\mu t} - 1)/\mu \) for "GFP."
from which two characteristic timescales [i.e., $1/(m+\mu)$ and $1/\mu$] can be identified. Evidently, the characteristic timescale for the expression of $\Gamma/$GFP is $1/(m+\mu)$. Because the matura-
tion constant $m$ is usually much larger than the growth constant $\mu$ (30, 34), the second term of $\Gamma/$GFP has a much larger co-
efficient than the first term, and thus, the second term domi-
nantly characterizes the expression of $\Gamma/$GFP with a critical timescale of $1/\mu$. Therefore, we approximate the critical time to induce cells to reach steady-state fluorescence as

\[
t_{\text{induce}} \approx \frac{1}{\mu}.
\]

Based on the known parameters for IPTGRCV/GFP cells encapsu-
lated in hydrogel at a typical distance of $L$ (0.7 mm) from
the environment, we can estimate the critical timescales of diffusion and induced to be 7.5 and 140 min, respectively. The induction of cells takes a much longer time than the transportation of inducers, which is consistent with the full model (Fig. 5 D and E). In the coupled diffusion–induction timescale is 2.4 h, which is also in good agreement with the full model's prediction (Fig. 5E).

The above analysis can provide a few guidelines for the design of future living materials. To design living materials and devices with faster responses, we need shorter times for both $t_{\text{diffusive}}$ and $t_{\text{induce}}$. To decrease $t_{\text{diffusive}}$ (Eq. 5), one can (i) reduce the thickness of the hydrogel, (ii) increase the diffusivity of inducer in the hydrogel, and (iii) increase the inducer concentration in the environment. However, to decrease $t_{\text{induce}}$, one can design cells with higher maturation constants or growth constants and add negative feedback into genetic circuit (31).

Conclusions

We have integrated genetically engineered cells as programmable functional components with stretchable, robust, and biocompatible hydrogel–elastomer hybrids to create a set of stretchable living materials and devices. These living materials and devices can be programmed with desirable functionalities by designing the genetic circuits in the cells as well as the structures and micro-

patterns of the hydrogel–elastomer hybrids. Moreover, we developed a quantitative model that accounts for the coupling between physical and biochemical processes in living materials. We further identified two critical timescales that determine the speed of response of the living materials and devices and provide guidelines for the design of future systems. This work has the potential to open technological avenues that capitalize on ad-
vanced in synthetic biology and soft materials to implement stretchable, wearable, and portable living systems with important applications in the monitoring of human health (1) and envi-
ronmental conditions (35) and the treatment and prevention of diseases (2).

Materials and Methods

All details associated with the materials, fabrication steps, and strain engi-
eering appear in SI Text. Note that all procedures involving human subjects conformed to the guidelines for protecting the rights of human subjects and were approved by the Massachusetts Institute of Technology Committee on the Use of Humans as Experimental Subjects (COUHES protocol no. 1701827491). All subjects provided informed consent.

Briefly, different cell strains were picked from overnight growth on LB plates and cultured in LB with 50 μg/mL carbenicillin at 37 °C. Cell cultures were infused into the patterned cavities between hydrogel and elastomer by metallic needles (Nordson EFD) through the hydrogel layer (Fig. S1). The holes induced by cell injection were sealed with small amounts of fast-curable pregel solution. The cell-contained device was washed with PBS three times followed by immersing the device in LB broth with carbe-

nicillin and inducer(s) at 25 °C.

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