3D printing has been intensively explored to fabricate customized structures of responsive materials including hydrogels, liquid-crystal elastomers, shape-memory polymers, and aqueous droplets. Herein, a new method and material system capable of 3D-printing hydrogel inks with programed bacterial cells as responsive components into large-scale (3 cm), high-resolution (30 μm) living materials, where the cells can communicate and process signals in a programmable manner, are reported. The design of 3D-printed living materials is guided by quantitative models that account for the responses of programed cells in printed microstructures of hydrogels. Novel living devices are further demonstrated, enabled by 3D printing of programed cells, including logic gates, spatiotemporally responsive patterning, and wearable devices.

3D printing is an emerging technology to fabricate customized structures of responsive materials that have found applications in areas as diverse as drug delivery, tissue engineering, soft actuators, and adaptive buildings. Existing stimuli-responsive materials for 3D printing include hydrogels, liquid-crystal elastomers, shape-memory polymers, and aqueous droplets. In nature, highly organized, 3D, multicellular living systems can perform complex cellular functions over time, such as cell cooperation, competition, proliferation, and apoptosis, in response to numerous spatiotemporally varying signals. Recent developments in synthetic biology have further enabled programing of living cells to accurately respond to various stimuli such as heat, light, and chemicals. 3D-printed architectures of programed cells will not only mimic highly organized, time-evolving biological constructs, but also provide new functions as living responsive materials and devices. However, the potential benefits of harnessing programed cells as active components in 3D printing have not been well explored due to challenges of integrating programed cells into macroscopic structures with high precision while maintaining their viability and responsiveness.

Here, we report a new method and material system capable of printing programed bacterial cells into large-scale (3 cm) high-resolution (30 μm) living networks that accurately respond to signaling chemicals in a programmable manner. The design of 3D printed structures is guided by quantitative models that account for the responses of programed cells in printed microstructures. We further demonstrate novel living devices enabled by 3D printing of programed cells, including logic gates, spatiotemporally responsive patterning, and wearable devices.

The design principle for 3D printing of living materials and devices illustrated in Figure 1a. On one hand, living cells as responsive components can be genetically engineered to possess various functions, including biosensing, drug production, chemical secretion, and programed cell death. On the other hand, the architectures of living materials and devices can be programed by 3D printing, including the microstructures of the hydrogel matrices, and the distribution of cells and chemicals within the matrices. We can simulate the chemical diffusion and cell reaction in the 3D-printed living networks and predict the behaviors of living materials under various conditions. In turn, the spatiotemporal responses of cells and matrices can provide rational feedback and guidance for the design of living materials and devices, thus enabling the programmable customization of living materials with predictable responsiveness.

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**3D Printing of Living Responsive Materials and Devices**

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3D printing has been intensively explored to fabricate customized structures of responsive materials including hydrogels, liquid-crystal elastomers, shape-memory polymers, and aqueous droplets. Herein, a new method and material system capable of 3D-printing hydrogel inks with programed bacterial cells as responsive components into large-scale (3 cm), high-resolution (30 μm) living materials, where the cells can communicate and process signals in a programmable manner, are reported. The design of 3D-printed living materials is guided by quantitative models that account for the responses of programed cells in printed microstructures of hydrogels. Novel living devices are further demonstrated, enabled by 3D printing of programed cells, including logic gates, spatiotemporally responsive patterning, and wearable devices.
Ink design is critical to ensure the viability and responsiveness of cells in living networks. Our ink system for 3D direct ink writing is based on a well-dispersed mixture of photoinitiator and polymeric micelles in water, with the addition of certain engineered bacterial cells, signaling chemicals, and nutrients. The components serve different purposes in the printing ink: Pluronic F127 diacrylate (Pluronic F127-DA) micelles provide ideal rheological behaviors required for direct ink writing, and the packing of Pluronic F127-DA micelles in the ink leads to a physically crosslinked hydrogel after printing\(^\text{[19]}\); Irgacure 2959 as photoinitiator allows postphoto-crosslinking of the living structures after printing; engineered bacterial cells are programed to sense the signaling chemicals and respond with fluorescence and chemical secretion; signaling chemicals, including N-acyl homoserine lactone (AHL), isopropyl β-D-1-thiogalactopyranoside (IPTG), rhamnose (Rham), and anhydrotetracycline (aTc), are allowed to diffuse in the matrices and interact with different cell types; LB broth is used as the nutrient to maintain the viability and functionality of cells presented within matrices. Bacterial cells used in this study are engineered with different responsive functions: GFP\(^+\) strain expresses green fluorescence protein (GFP) constitutively; AHL/GFP\(^+\), Rham/GFP\(^+\), or IPTG/GFP\(^+\) strain produces GFP when chemical inducer AHL, Rham, or IPTG is received by the cognate cell strain, respectively; aTc/AHL\(^+\) strain secretes AHL when aTc is detected by the cell strain; AHL/GFP\(^−\) strain can repress GFP production in cell when AHL is detected. In all, the living ink system provides a nourishing, biocompatible environment for cell survival and function (Figure 1b,c).

The 3D printing procedure for living materials consists of two steps: (i) direct writing of multiple hydrogel inks with various types of cells and chemicals (Figure 1b), and (ii) ultraviolet (UV) curing of the printed constructs (Figure 1c). During the direct ink writing, the nozzle diameter we adopt ranges from 30 to 200 \(\mu\)m, the nozzle feed rate is fixed at 5 mm s\(^{-1}\), and the printing pressure is set as 90 kPa. We show that the mechanically reversible, rapidly responsive dissociation/association of Pluronic F127-DA micelles in water (Figure 1b) endows the ink with a strong shear-thinning effect during printing (Figure S2a, Supporting Information), and a plateau storage modulus (\(G'\)) orders of magnitude higher than loss modulus (\(G''\)) after printing (Figure S2b, Supporting Information). Thus, the Pluronic F127-DA micelles provide suitable rheological behaviors for 3D living printing. During UV irradiation, the printed sample is exposed to 1 J m\(^{-2}\) UV for 5 min.
The photo crosslinking is carried out under a humid nitrogen atmosphere to prevent hydrogel dehydration and oxygen inhibition of polymerization. The reactive end groups in Pluronic F127-DA enable the formation of covalent crosslinks among micelles after UV crosslinking (Figure 1c). The resultant hydrogel is mechanically robust and stretchable, with shear modulus of 50 kPa, toughness of 77 J m$^{-2}$, failure stretch of 162%, and fast recovery of elasticity within 1 min (Figure S3, Supporting Information). The mechanical robustness and structural integrity of hydrogel matrices can potentially enable applications of living devices that require mechanical reliability under cyclic deformation and robust integration with other engineering materials.$^{[20–22]}$

To systematically evaluate the printability of the living ink, we vary the printing pressure and Pluronic F127-DA concentration in the ink, while fixing other parameters including nozzle diameter, nozzle feed rate, and ink compositions (e.g., cells, nutrients). We find the ink can be printed into 3D structures when the concentration of Pluronic F127-DA is between 18 and 36 wt% and the pressure is above a certain threshold, as shown in Figure 2a. When Pluronic F127-DA concentration is below 18 wt% or above 36 wt%, the ink cannot be printed due to liquid spreading or nozzle clogging, respectively; and when the printing pressure is below the threshold value, the ink cannot flow out of the nozzle (Figure 2a). In addition, we find that the printability of the ink is independent of nozzle dimensions (30–200 µm) for the ink composition used in this study (Figure 2a; Figure S4, Supporting Information).

To demonstrate the capability of printing large-scale and complex structures with multimaterial inks, various 3D architectures are printed (Figure 2b–f; Video S1, Supporting Information), including a cuboid, a pyramid, a dome, and hollow pyramids. The overall dimensions of the printed architectures can reach up to 3 cm. The first three cases (cuboid, pyramid, and dome, Figure 2b–d) show the capability of printing any solid constructs. In addition, the last two examples (hollow pyramids, Figure 2e,f) show that overhanging beams can be printed and the printed structures can be self-supporting due to the high yield stress of the ink materials (Figure S2b, Supporting Information). The multiple materials (red and green inks, Figure 2f) printed in selective regions of the last hollow pyramid demonstrate that our 3D printing technique is capable of integrating various types of cells and chemicals into a single construct. In addition, the 3D printing method can achieve very high spatial resolution as demonstrated in the printed grid microstructures with different local dimensions (Figure 2g–j). With encapsulated GFP+ bacterial cells, the grid microstructures of hydrogel matrices are clearly visualized under confocal microscope, with feature sizes varying from 200 µm (Figure 2g), 100 µm (Figure 2h), 50 µm (Figure 2i), and 30 µm (Figure 2j).
Therefore, we can create complex macroscopic structures (3 cm) with microscopic precision (30 µm) through the 3D multimaterial printing method. The 3D printing method and the ink materials can also give very high viability of bacterial cells in the printed living networks, due to the following reasons. First, all ink components including Pluronic F127-DA and Irgacure 2959 are biocompatible.[19,23] Second, the fabrication processes, including microextrusion (i.e., 90 kPa pressure) and UV irradiation (i.e., 1 J m⁻² UV for 5 min) are mild for the encapsulated bacterial cells.[3] Bacterial cells with protective cell walls are able to survive relatively harsh conditions (such as shear force during extrusion and UV exposure during crosslinking) compared with more susceptible mammalian cells.[24] Third, hydrogel matrices can supply water and nutrients to cells, and ensure diffusion of signaling chemicals in the encapsulated multicellular system,[10] while fine features (30–200 µm) of the matrices allows for sufficient gas permeation.[25] To test cell viability in the hydrogel matrices, we use live/dead stain and fluorescence imaging for the cells in hydrogel matrices (200 and 100 µm in feature sizes). As shown in Figure S5 (Supporting Information), bacterial cells are highly viable (i.e., viability >95%) 24 h after printing. Therefore, the 3D printed microstructures provide a biocompatible, mechanically soft, and robust matrix that supplies nutrients and water for genetically engineered microorganisms, and ensures the successful operation of living materials over time.

The 3D printing of living materials and devices enables us to explore novel functions, including logic gates, spatiotemporally responsive patterning, and wearable devices. With the rapid development in synthetic biology, single cells have now been engineered to have new functions such as logic, memory, oscillator, and classifier.[9,13,26] To enable multicellular logic and guide signaling transmission for cell–cell communication, we print multiple types of cells and chemicals into 3D architectures and allow communication between different cell types to follow the well-defined networks of hydrogel matrices, thus achieving logic gates in the 3D-printed living materials and devices (Figure 3a,b). Each cell in the structure performs a simple computational operation. However, combined with their spatial distributions in the 3D architectures, the interactions among different cell types and chemicals in different regions can induce the emergence of informative patterns and achieve complex logic operations. For example, to perform single-input logic gates achieved by multiink 3D printing of living materials. a) Schematic illustration of a 3D-printed living scaffold that can function as a single-input and single-output (SISO) Boolean logic gate. b) Schematic illustration of a 3D-printed living scaffold that can function as a double-input and single-output (DISO) Boolean logic gate. c) Experimental results of logic gates, indicated by green fluorescence in one layer of the printed hydrogel containing the output. The absence or presence of active components is denoted as 0 or 1 for inputs, while the absence or presence of GFP production is denoted as 0 or 1 for outputs. The input hydrogel layer remains dark no matter if it is 0 or 1, while the output hydrogel layer can be either green (1) or dark (0). The symbols (insets) show the inputs, computation, and outputs for each condition (scale bars in (c), 500 µm). Buffer gate input: AHL in hydrogel, output: AHL/GFP⁺ bacterial cells in hydrogel. NOT gate input: AHL in hydrogel, output: AHL/GFP⁻ bacterial cells in hydrogel. AND gate inputs: αTc in hydrogel, αTc/AHL⁺ bacterial cells in hydrogel. OR gate inputs: AHL in hydrogel, Rham in hydrogel, output: a mixture of AHL/GFP⁺ bacterial cells and Rham/GFP⁺ bacterial cells in hydrogel. NAND inputs: αTc in hydrogel, αTc/AHL⁺ bacterial cells in hydrogel, output: AHL/GFP⁻ bacterial cells in hydrogel.
and single-output (SISO) logic computation, we print orthogonally distributed hydrogel filaments; the rows contain chemicals which act as input, while the columns contain cells which act as output (Figure 3a,c). For double-input and single-output (DISO) Boolean logic gate, we print three types of crossed hydrogel filaments: the top ones contain input a, the bottom ones contain input b, and the columns in between contain cells which act as output (Figure 3b,c). The absence or presence of active components is denoted as 0 or 1 for inputs, while the absence or presence of GFP production is denoted as 0 or 1 for outputs. The input hydrogels remain dark no matter if they are 0 or 1, while the output hydrogel columns can be either green (1) or dark (0). As shown in Figure 3c and Table S1 in the Supporting Information, Buffer, NOT, AND, OR, and NAND gates are implemented by depositing different cells and chemicals in the constructs. In the Buffer gate, the output (AHL/GFP+ cells) is originally dark in the absence of AHL, while the presence of AHL can activate fluorescence in cells. In the NOT gate, the output (AHL/GFP− cells) is originally green in absence of NOT input, while the presence of an inhibitor (AHL) can repress GFP synthesis. In the AND gate, the AHL/GFP+ cells become green only when both input a (aTc) and input b (aTc/AHL+ cells) are present. In the OR gate, the output contains two cell types, AHL/GFP+ cells and Rham/GFP+ cells, and it turns on when either AHL or Rham is present. In the NAND gate, aTc and aTc/AHL+ cells act as two inputs which can induce the disappearance of green fluorescence in the output (AHL/GFP− cells). While logic gates that use nonliving chemicals or hydrogels have been reported,[27,28] here we demonstrate the first set of 3D-printed living logic gates using programed cells in hydrogels. These intricate structures, enabled by 3D multimaterial printing, provide well-organized, higher-level living cell networks that integrate multiple input signals for intelligent applications, including decision making and actuation in complex environments.[29]

Next, we demonstrate the capability of 3D-printed living materials to achieve spatiotemporally controlled patterning, owing to well-defined spatial distribution and time evolution in the living devices (Videos S2 and S3, Supporting Information). We choose two exemplary living patterns: a 1D pattern composed of cell parallel lines and a chemical perpendicular line (Figure 4a), and a 2D pattern composed of cell concentric rings with chemical radial lines (Figure 4e). The encapsulated cells we use in this particular experiment is AHL/GFP+ and the encapsulated chemical is AHL. For the 1D-living pattern, the set of experimental images (Figure 4b) reveal the spatiotemporal evolution of fluorescence in the living materials over 8 h. Similarly, the spatiotemporal fluorescence in the circular 2D-living pattern over 4 h is shown in the experimental images (Figure 4f). In both cases, the frontline of responded cells (i.e., turning fluorescent) within the hydrogel propagates along the paths of signaling chemical diffusion, and the fluorescence intensity of responded regions increases over time. We further develop a coupled diffusion–induction model that accounts for the chemical and cellular behaviors to predict the spatiotemporal responses of the living patterns. The prediction of the spatiotemporal patterns of the 3D-printed living materials relies on the understanding of two processes: (i) diffusion of signaling chemicals in hydrogel matrices and (ii) induction of encapsulated bacterial cells by signaling chemicals. The governing equations for these two processes are adopted from classical models[16,17] and given in the Supporting Information. To solve the governing equations, numerical models are developed for the living patterns using COMSOL Multiphysics with typical parameters listed in Table S2 in the Supporting Information. As shown in Figure 4b–d,f–h, the good agreement between experiments and simulations validates the predictability of our models. Therefore, the design of 3D living materials and devices can be rationally guided by such predictive models that account for the responses of cells and hydrogel matrices.

Besides the relatively planar structures with height of 400–600 µm shown in Figures 3 and 4, we further present a multilayer living responsive structure with height of 3.6 mm as illustrated in Figure 5a (Supporting Information). We print hydrogel inks that contain AHL and AHL/GFP+ cell in different regions of the 3D structure. Over time, the AHL molecules will diffuse from the AHL hydrogel region into the hydrogel region that contains AHL/GFP+ cells. Once the cells are induced (>4 h after printing), the hydrogel region with cells in the living structure will become fluorescent (Figure S6b–c, Supporting Information).

The 3D printing of programed cells enables us to explore novel functions of living devices otherwise unachievable with structural design, printing fabrication, or genetic engineering alone. A living tattoo is designed to integrate a collection of multiple chemical-sensing cells (responsive to AHL, Rham, or IPTG) printed on the surface of a bilayer elastomeric sheet, and carefully attached to human skin (Figure 5a). The thin (≈75 µm), flexible, transparent, and gas permeable bilayer elastomeric sheet is fabricated by successive spin-coating deposition of two silicone elastomeric layers (Figure S7, Supporting Information), where the upper layer (Sylgard 184) is mechanically robust and flexible, and the lower layer (Silgel 613) is skin-adhesive which gives robust bonding to skin via van der Waals forces alone without the need for any additional fixtures or tapes (Figure 5a). The living device does not impose any mass loading on the skin nor mechanical constraint of the body motion.[29] As shown in Figure 5b, the skin can deform in different modes (i.e., stretching, compression, and twisting) freely and reversibly without any detachment of the living tattoo. Moreover, robust bonding between the hydrogel and the elastomer sheets and their mechanically soft nature prevents detachment (Figure S7, Supporting Information).[20,30] Further, the elastomer layer effectively reduces water loss from the printed hydrogel, and thus maintains cell viability and device functionality.[20] To examine practical applications of the living tattoo, three signaling chemicals (e.g., AHL, Rham, or IPTG) are smeared on the skin, respectively. When the chemicals are received, the adhered tattoos exhibit green fluorescence in corresponding 3D printed patterns. The monitoring of different chemicals by the living tattoo device on the skin is shown by experimental results (Figure 5c).

In summary, we report a new paradigm in 3D printing by using genetically programed cells as active components to create living materials and devices. This can be a new 4D-printing approach to produce 3D structures with time-evolving properties.[11–18] We elaborate design principles and general methods to print large-scale high-resolution living materials that are capable of integrating engineered cells
into hydrogel constructs while maintaining high cell viability and ability to receive and process signals in a programmable manner. Novel applications enabled by 3D living printing of programed living cells are demonstrated, including logic gates, spatiotemporally responsive patterning, and wearable devices. The integrative technology of 3D living printing has the potential to be used as a general platform where a range of genetically programed cells (for example, cells with therapeutic production), matrices (for example, biodegradable hydrogels), and structures (for example, a cartilage shape) can be applied to design more customized living materials and devices with predictable dynamic functionalities. For instance, it is intriguing to envision a robust and personalized implant in which different cell types are programed to monitor inflammatory biomarkers and release growth factors to promote angiogenesis. New ingestible devices based on our 3D printing of living materials...
may be able to modulate the gut microbiota and treat microbe-mediated disease such as obesity and diabetes.\(^{[12]}\) Overall, our 3D printing of living materials and devices opens new avenues toward real-world applications, such as on-demand medicine, biotechnology, bioremediation, and bioenergy via cell programming, structural design and direct ink writing technologies.

**Experimental Section**

The general procedures for creating 3D-printed living materials and devices involved the preparation of ink containing Pluronic F127-DA, photoinitiator, LB broth, antibiotic, chemical inducer, bacterial cell pellets, and deionized (DI) water. 3D architectures were printed at room temperature in air, and ultraviolet crosslinked after print completion. Samples were kept in a humid chamber. Unless otherwise specified, the chemicals used in the current work were purchased from Sigma-Aldrich and used without further purification.

**Ink Preparation:** The ink formulation for all experiments contained Pluronic F127-DA as polymer matrices, Irgacure 2959 as photoinitiator, LB broth (Miller) as nutrient, DI water, together with certain chemical inducers, antibiotics (i.e., carbenicillin), and/or bacterial cell pellets. Pluronic F127-DA was synthesized according to a reported protocol.\(^{[13]}\) Briefly, 25 g of Pluronic F127 were dissolved into 80 mL of toluene at 60 °C under nitrogen with a condenser with circulating cold water. After 4 h, the solution was cooled down to room temperature, and then to 0 °C in an ice bath. 1.2 mL of trimethylamine was added to

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**Figure 5.** 3D-printed living tattoo for chemical detection on human skin. a) The design of the living tattoo. The tattoo is printed as a tree-like pattern on a thin elastomer layer, which is then adhered to human skin. Hydrogels with different colors illustrate the different types of cells encapsulated. Inset: Schematic illustration of living sensors embedded in the tattoo, which can respond to different chemicals by emitting fluorescence. b) The living tattoo on skin in different states: stretched (left), compressed (middle), and twisted (right). Food dyes are added to facilitate visualization of the hydrogel pattern in (a) and (b). c) The response of the living tattoo on the skin smeared with Rham (left), IPTG (middle), or AHL (right).
the solution, followed by the dropwise addition of 0.5 mL of acryloyl chloride. The mixture was stirred overnight. Afterward, the precipitated byproduct was filtered away, and the solution was concentrated by rotary evaporator. The white product was precipitated from the viscous solution by adding hexane. Through a second filtration step and drying in a fume hood for 48 h, the Pluronic F127-DA product was obtained. The synthesized Pluronic F127-DA at 27 wt% concentration and Irgacure 2959 at 0.2 wt% were dissolved in DI water in the dark at 4 °C. Then, 0.25 wt% LB broth powder and 50 µg mL⁻¹ carbenicillin were added to the mixture. Different active components, including chemical inducers (e.g., 100 × 10⁻⁶ M AHL, 1 × 10⁻⁴ M IPTG, 200 ng mL⁻¹ aTc, and 12 × 10⁻₃ M Rham) and cell pellets (e.g., AHL/GFP+ cell, Rham/ GFP+ cell, tAHL+ cell, and GFP+ cell), were introduced in different ink systems. The mechanical sensitivity in the printing ink was ~10⁻⁶ cells per mL. The freshly prepared inks were loaded into a UV-blocking 5 mL syringe barrel (EFD Nordson), and allowed to deam at 4 °C for 1 h.

**Printing and Crosslinking:** The 3D living materials and devices were fabricated using a three-axis robotic deposition stage (Aerotech) by printing a 3D structure onto a glass slide (Corning) or a chambered coverglass (Lab-Tek) covered with a hydrophobic coating (Rain-X). The structures were designed using commercially available software including Solidworks (Dassault Systèmes) or CADfusion (Aerotech), and translated into code using a custom Python script. The prepared inks were housed in the 5 mL syringe barrels, which fitted with nozzles ranging in size from 30 µm (World Precision Instruments), 50 µm (Fisnar), 100 µm (Fisnar) to 200 µm (EFD Nordson). To achieve stable and optimal printing, we choose 90 kPa of air pressure (Ultimus V, Nordson EFD) as printing pressure, and 27 wt% Pluronic F127-DA in the ink for all experiments, where the printed hydrogel filaments are continuous and uniform, and their diameters are nearly the same as the nozzle diameters. After deposition, the physically crosslinked micelles was then chemically crosslinked by UV irradiation in a UV chamber (365 nm in wavelength, 1 J m⁻²; UVP CL-1000) for 5 min, and the chamber was saturated with water vapor and nitrogen. After curing, the 3D structures were kept in a humid chamber.

**Rheological Characterization:** Ink rheology was measured using a controlled stress rheometer (AR-G2, TA Instruments) with a 40 mm diameter 2° cone-plate (54 µm gap) and parallel-plate geometry (250 µm gap). The rheology of chemically crosslinked hydrogel was measured using the same instrument with a 40 mm diameter parallel-plate geometry (3.2 mm gap). Shear viscosity measurements of ink were carried out in steady-state flow mode. The storage shear moduli of ink were measured using an oscillatory stress sweep at a frequency of 1 Hz. To study the time-dependent mechanical properties of physically crosslinked ink and chemically crosslinked hydrogel, the rheometer was used to measure the storage and loss shear moduli at different frequencies of oscillating strain (γ = 0.01J). Measurements were carried out at 25 and 4 °C using an aqueous solvent trap to mitigate drying effects.

**Living Tattoo Fabrication:** The bilayer elastomeric sheet was fabricated by successive spin-coating deposition of two silicone elastomers, Sylgard 184 (Dow Corning) and Silgel 613 (Wacker). Sylgard 184 was first spin-coated on the glass at 1500 rpm for 60 s, and then cured at 70 °C for 1 h. Then, Silgel 613 was spin-coated on top of the Sylgard 184 at 1500 rpm for 60 s, and then cured at 70 °C for another 1 h. The elastomer surfaces were thoroughly cleaned with methanol and DI water, and completely dried with nitrogen gas before the benzophenone treatment. Thereafter, a benzenophene solution (10 wt% in ethanol) was applied onto the elastomer to evenly cover the entire elastomer surface for 1 min at room temperature. Then, the elastomer was washed with methanol three times and completely dried with nitrogen gas. The multiple hydrogel inks, which contain different cell sensors, were printed directly on the elastomer with 200 µm size nozzles, followed by 5 min UV curing, which led to the chemically crosslinked hydrogel and the robust bonding between hydrogel and elastomer. Lastly, we carefully peeled off the living tattoo from the glass slide, and adhered it on human skin for further testing.

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.

**Cell Viability Assay:** 24 h after fabrication, the cell-laden hydrogels were peeled off from glass slides, and washed with 0.85% NaCl solution. Using the LIVE/DEAD BacLight bacterial viability kit, we stained the cells with SYTO 9 (5.01 × 10⁻⁴ M; Invitrogen) for live cells and propidium iodide (30 × 10⁻⁶ M; Invitrogen) for dead cells. 15 min after staining, fluorescent imaging of samples (n = 3) was carried out using an epifluorescence microscope (Nikon Eclipse LV100ND). Live and dead cell counts were obtained using the 3D objects counter plugin in ImageJ software.

**Numerical Modeling:** We developed a 2D finite-element model to simulate the chemical diffusion and cell induction in hydrogel patterns using COMSOL Multiphysics. Time-dependent transport of diluted species (tds) module as selected for the study. Parameters are shown in Table S2 (Supporting Information), and the geometries were the same as those in the experiments.

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**Code Availability:** G-code (print path) for all structures printed in Figures 2–5 is available on request.

## Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

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