ORIGINAL ARTICLE

bioPrint: A Liquid Deposition Printing System for Natural Actuators

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Abstract

This article presents a digital fabrication platform for depositing solution-based natural stimuli-responsive material on a thin flat substrate to create hygromorphic biohybrid films. *Bacillus subtilis* bacterial spores are deposited in the printing process. The hardware system consists of a progressive cavity pump fluidic dispenser, a numerical control gantry, a cooling fan, a heating bed, an agitation module, and a camera module. The software pipeline includes the design of print patterns, simulation of resulting material transformations, and communication with computer hardware. The hardware and software systems are highly modularized and can therefore be easily reconfigured by the user.

Biological Actuators and Digital Fabrication

RECENT STUDIES IN MATERIAL SCIENCE and mechanical engineering have investigated new classes of materials that output dynamic shapes. Shape memory alloys, shape memory polymers, electroactive polymers, and pneumatic soft actuators have been introduced as emerging material platforms for designers with which to create applications beyond medical and robotic fields. Looking to nature for inspiration, the wilting of flowers and the opening of fallen pinecones have, among others, served as inspiration for the design of biological sensors and actuators. The utilization of such mechanisms from nature via the integration of natural microorganisms into design and engineering processes has gained increasing interest among scientists and engineers. Some research projects, including ours, have explored the use of natural microorganisms as sensors and actuators.

To fully explore the potential of these materials, digital fabrication processes and pipelines are presented.[1,2] The ability to arrange material structures at different scales allows us to preprogram material transformations under certain stimuli.[3,4] In particular, bioprinting represents an emerging technology for the construction and fabrication of biomaterials for research in bioengineering. Most of the current bioprinting technologies are inkjet and extrusion based. Inkjet printers enable precise control over the location of droplets and thus provide great flexibility to the user. For details about the hardware technology involved in this approach, numerous research articles are available[5,6] that demonstrate a step-by-step approach for integrating an inkjet head with a CNC router. Despite the significant progress in inkjet-based bioprinting, this technique still faces some limitations. One of the main restrictions is the maximum printable viscosity of bioink. Cell aggregation and sedimentation in the cartridge and clogging of the nozzles are additional limitations associated with this technique.[7]

The method that uses a pressure- or extrusion-based printing system has been applied for some time. Extrusion-based-bioprinting consists of a pneumatic or mechanical fluid-dispensing system feeding an extruder on an automated robotic gantry.[8,9] This method overcomes the viscosity limitation and clogging problem present in the inkjet system. However, there is often a trade-off in terms of the precision of printing.

In this article, we introduce an open hardware and software platform for printing suspended *Bacillus subtilis* spores to pattern humidity-responsive nanosensors and actuators for local material shape transformation.

Design Space with Micro–Macro Biology

New and emerging engineering and computational components in the study of biology are of increasing relevance for the design community. We examine the biological performance at different scales and identify a novel design space that bridges the micro (cellular level) and macro (human level) scales. While synthetic biology primarily focuses on the nanoscale engineering of DNA, biological science

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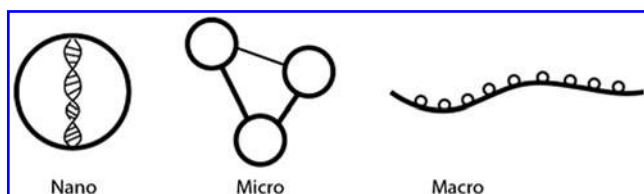
FIG. 1. Biological formation and transformation across scale.

expands the size of the material into the micron scale for achieving cell–cell interactions. In this article, we focus on the mechanical properties of bacterial spores: the controlled deposition of microsized spores on transformable macro-surfaces. This approach leverages the advantages of natural microorganisms while achieving a human-scale interaction level (Figure 1).

Development of Biohybrid Films

Introducing B. subtilis spores as nanoactuators

Humans have continued largely replacing animal-driven tools (e.g., horse mill) with machine-driven tools (e.g., steam engine, electric motor, and internal combustion engine). The latter have the advantage of being more precise and controllable. In a rare move opposing this trend, organic actuators found in nature have been studied in the context of mechanical transformation.[10] The natural actuator we used in this printing process was the *B. subtilis* endospore, which is the sporulation state the *B. subtilis* bacterium assumes under exposure to extreme conditions. Earlier research showed that a dormant *B. subtilis* spore can be used to bend and release a thin sheet substrate, due to this spore's cortex layer, which is hygroscopic in nature.[6] The resulting mechanism has an extremely high energy density (10.6 MJ/m^3) and is easy to assemble. Dormant spores can also survive and maintain hygromorphic behavior without any nutritional supply for extremely long periods of time (over thousands of years).[11]

Figure 2 is a scanning electron microscopy scan showing a *B. subtilis* endospore in its dry state. On average, spores are 1 μm in length.

Using a natural microorganism as actuator has several distinctive advantages; for example, it is electronicity-free, is biocompatible, lacks wires and tubes, delivers silent actuation, holds potential biological synthesis, is capable of self-reproduction, and presents flexibility in terms of deposition as a liquid form.

Spores preparation in wet lab

We adapted the classic process of *Bacillus* spore cultivation and developed our own repeatable process for harvesting *B. subtilis* natto spores in a standard wet lab under the conditions of biosafety level one, which is the lowest biosafety level requirement that current biohacker spaces must meet. A standard, relatively inexpensive setup and equipment was used, including a freezer, pipettes, shaker, centrifuge, lab glassware, and plastic ware.

The entire process included a spore culture from mother cells, as well as spore purification and dilution (Figure 3). Both spores from *B. subtilis* natto and *B. subtilis* 168 (purchased from Bacillus Genetic Stock Center) were cultured and purified using the following method: Cells were inoculated from the cell bank in 40% glycerol, which is contained in a culture tube with 10 mL Difco™ sporulation medium (DSM) containing 10% (w/v) KCl, 1.2% (w/v) MgSO$_4$·7H$_2$O, 1 M NaOH, 10 mM Ca(NO$_3$)$_2$, 10 mM MnCl$_2$, and 1 mM FeSO$_4$ with an adjusted pH of 7.6. This tube was incubated in a shaker at 30°C with a shaking speed of 250 rpm for 3–6 hours. Once the optical density of the culture reached 0.4–0.6 at 600 nm, 1 mL culture medium was transferred to a 500 mL or 1 L shake flask containing a 100 or 200 mL medium at 30°C with a shaking speed of 250 rpm for another 4–7 days before harvesting. The culture samples were monitored by a microscope to examine the sporulation stages. When the percentage of spores in the total population reached >99% with trace amounts of vegetative cells, the cultures were filtered through a Buchner funnel on top of a Buchner flask in a
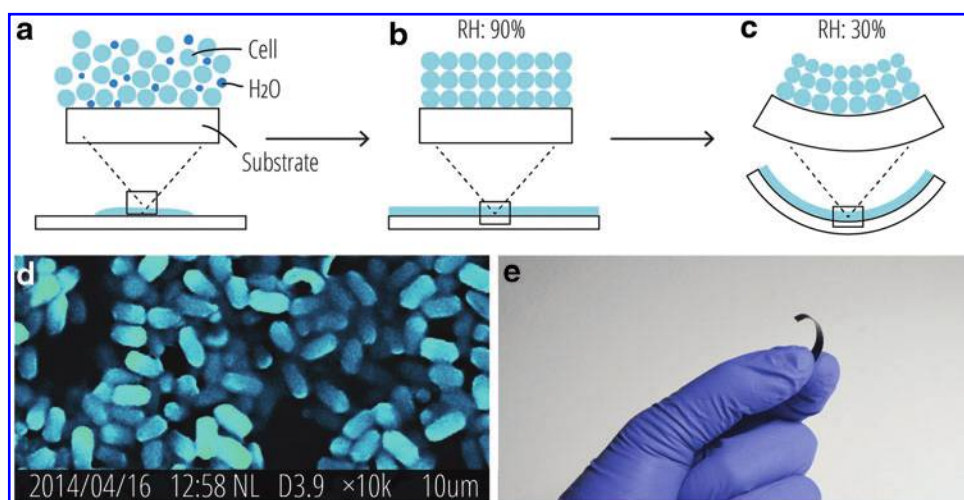

FIG. 2. Biohybrid film is a bilayer film made of *Bacillus subtilis* spore layer and another flexible inert film. (a) Deposition of the spore solution. (b) Biohybrid film at high relative humidity (RH). (c) Biohybrid film at low RH. (d) *B. subtilis* spores under scanning electron microscopy. (e) Biohybrid film. Color images available online at www.liebertpub.com/3dp
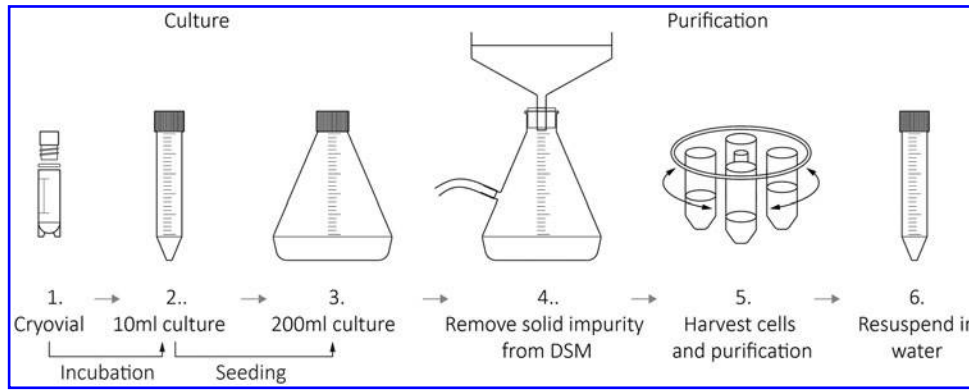
FIG. 3. Spores preparation process.

vacuum. Solid impurities were removed from the DSM. Then, the spore suspension was centrifuged at $3000 \times g$ for 10 min at 4°C. The spores settled at the bottom of the tube, with a brownish culture medium rising to the top. The medium was further removed by pipetting. After that, the spores were washed twice with water using the same centrifugation conditions to remove the trace amount of culture medium. Then, the spores were suspended in a certain amount of water with a final optical density of 35–40 (measured at 600 nm). This corresponded to a cell density of $5–6 \times 10^9$ colony-forming units/mL.

Development of biofilms

With the *B. subtilis* spore, we introduce the idea of developing biohybrid films that can transform as a result of changes in relative humidity. We obtained a composite biofilm by printing a spore–water solution onto the substrate layer and vaporizing the water content (Figure 2a). An ideal substrate material includes a 0.2-mm-thick latex, 0.3 mil Kapton, and 0.3 mil polyethylene terephthalate.

In previous research, we presented a series of explorations regarding the design of transformable biohybrid materials (Figure 4). By manually depositing a spore solution at a desired location on substrates, we were able to create simple bending structures. We also showed that more complicated bending structures can be achieved by laser cutting 2D patterns on a fully spore-coated biohybrid film. In this article, we introduce a method of integrating digital fabrication for more precise manufacturing in order to embed a certain level of programmability for achieving the desired transformations.

Although most of our previous studies had been produced using a pipetting process, we could reach only a certain level of complexity in terms of spore layout and distribution. The applications include ''living'' paint: as the paint is applied to scales on paper toys, the scales become transformable when people breathe on them (Figure 5).

FIG. 4. Examples of transformable geometric patterns.

The Printing System

To have high-precision control over the spore deposition process, we developed our own printing system, bioPrint. Compared with other bioprinters used in biolabs today, our printer has a number of distinct functions that have been customized for our specific needs: clogging is prevented through the use of a special progressive pump-based dispenser; the printer has fast movement; it prints with a relatively low resolution of a hundred microns rather than at a submicron resolution, since the application we focused on was at human scale; it does not need a controlled sterile environment, since we do not expect the spores to grow once the film has been produced.

bioPrint contains off-the-shelf hardware components and a software platform developed on top of open source plugins (Figure 6). bioPrint was designed with a few primary goals in mind: an easy work flow, starting from geometric design to G-code generation, to machine control to material fabrication; a high-precision deposition system for droplets ranging in width from 10 μm up to 5 mm; suitability for a large set of diverse user groups, including designers, artists, and scientific researchers; and safety and hygiene.

Hardware Platform

The hardware system includes a machine base, as well as modular components (Figure 7). It is built with off-the-shelf components and easily machinable parts.

Machine base

The machine base includes a standard three-axis CNC gantry platform, two mounting substrates for attaching modular components, and a central control system (Figure 6). In our demo system, we use a CNC kit (F8 version) from Zen Toolworks.[2] A higher-end CNC platform will help to increase the printing resolution. The mounting substrates are used to mount the central dispenser, as well as other configurable modular components. They have embedded magnets at certain locations for holding all modular components to the same place each time they are placed. The breakout control board supports up to five one-axis stepper motors, five input ports, and five extra output ports for accepting signals and sending commands to the modular components.

FIG. 5. Living paint. Spore solution as an active ink to actuate toy fish scales. Color images available online at www.liebertpub.com/3dp

Functional components

All the functional components have been designed with specific mechanical structures and magnet assemblies for easy plug–unplug and configuration actions. All the modules incorporate alignment magnets to ensure the same exact location of placement.

- The dispenser is the central component. It is a progressive cavity pump-based dispensing head (EcoPen 300 from ViscoTec-America Inc.) that enables droplet widths from $10 \mu m$ up to $5 mm$. The dispenser is controlled by the central control system; a customized G-code can turn the dispenser on and off on demand.
- Solution container. There are two types of solution containers: liquids that flow quickly under their own weight and that can be loaded into a gravity-based container without a cap; for solutions with higher viscosity, a closed container with controllable pneumatic pressure is used.
- Ventilation. Certain solutions solidify only when water or other chemicals evaporate. In such a case, a ventilation module with two speed-tunable fans can be placed on top of the printing platform.
- Mechanical stir. Noncolloidal substances have particles unstably suspended in a liquid. These materials are not soluble and can possibly form sediment. This problem becomes obvious when it comes to biological sample printing. Mechanical stirring is a useful approach for preventing the spore–water mixture from aggregating.
- Camera. We currently use a webcam to remotely track printing progress; however, more interesting work can be

done with a live video stream if computer vision techniques are adapted to recognize the parts or the region being printed. For example, an object can be detected and set as being the original location of the printing path.

Software Platform

In the software system, the workflow includes design, simulation, G-code generation, and firmware communication. Design, simulation, and G-code generation are conducted using a platform based on the Rhino and Grasshopper 3D modeling engines; a universal G-code sender is used to send the G-code to the machine. Since our targeted user base includes people with different levels of digital design and modeling skills, as well as different software features for designing different printing paths, we decided on the following software design strategy: a set of parametric tools based on the most commonly used printing patterns and customized with parameter sliders. Thus far, we have basic toolsets for handling 1D, 2D, and 3D structures; more customized variations can easily be developed on top of the current platform.

Tool path generation

Offsetting a line path. The printing path can be a group of lines that come from the offset of an existing open or closed geometry. The line gaps and the number of lines are adjustable (Figure 8).

Infilling a geometry. A user draws a closed curve to indicate the region for printing. The tool will generate the printing path to fill the region. The distance or line gap is
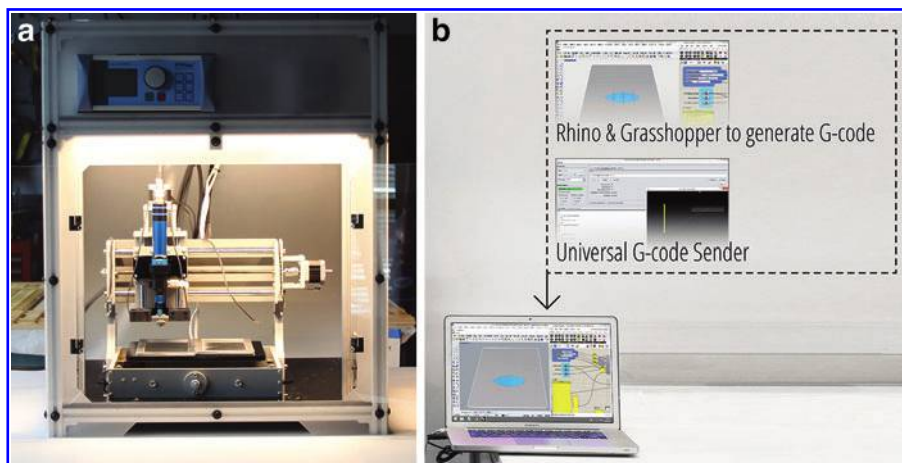

FIG. 6. bioPrint system. **(a)** Hardware design. **(b)** Software pipeline. Color images available online at www.liebertpub.com/3dp
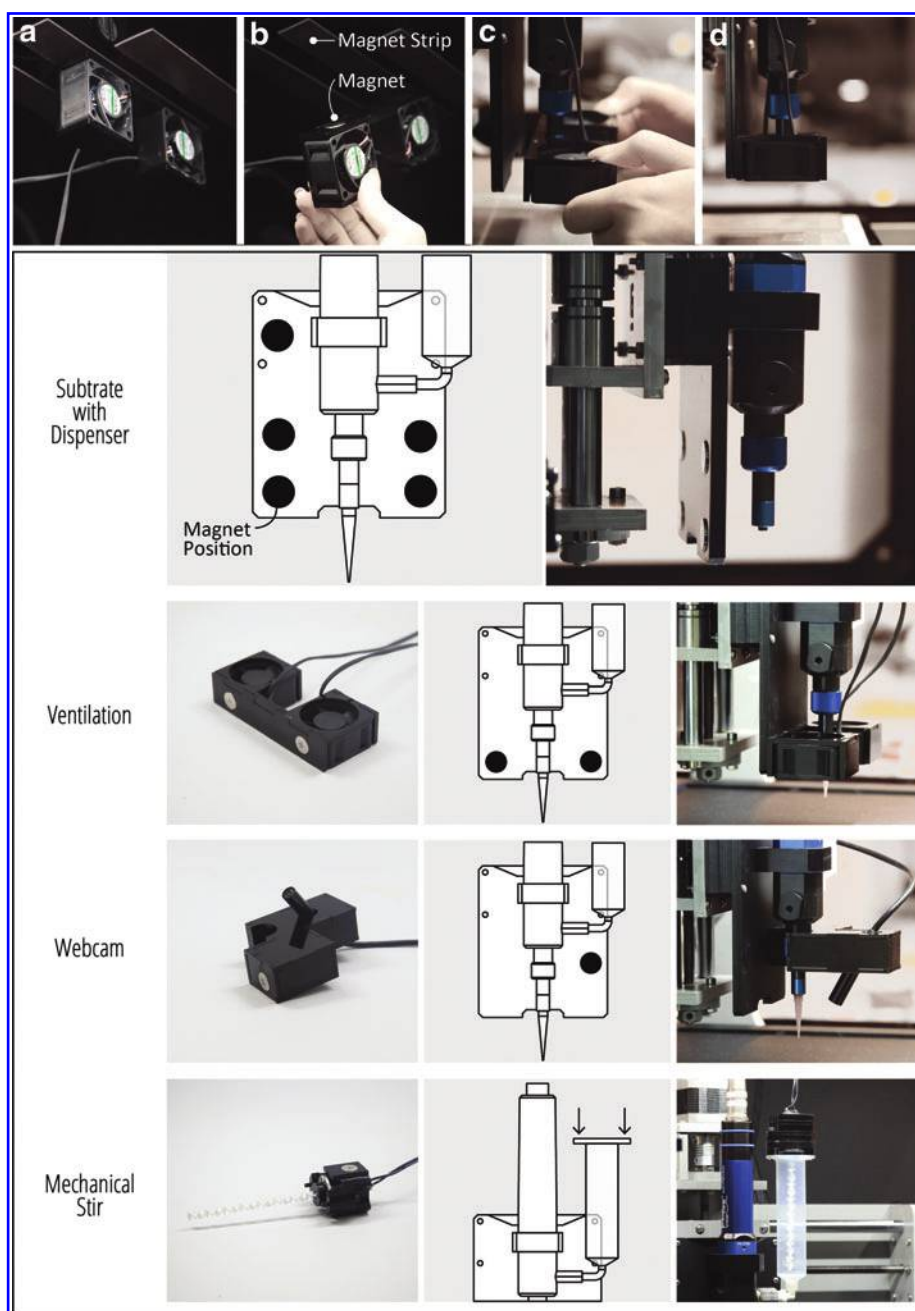
FIG. 7. Functional components include dispenser, solution container, ventilation module, mechanical agitation module, and camera. **(a)** The ventilation module is magnetically mounted for storage; **(b)** it can be taken off easily by hand; **(c)** the module can be magnetically re-allocated and mounted on top of the machine; **(d)** the module is in working mode. Color images available online at www.liebertpub.com/3dp

adjustable using a slider. If the printing needs to be repeated multiple times, the duration and waiting time in between patterns can be adjusted (Figure 9).

Simulation. Simulation is strongly related to the specific material's responsiveness, as well as to the printing structure. We implemented a tool that simulates hinge folding-based transformation when an actuator material is printed on a bilayer structure. A more mechanically sophisticated simulation can be developed to demonstrate the feasibility of an integrated system using the same software platform (Figure 10).

In the back end of our software, modeling methods have been incorporated for simulating material behavior. At the core, these computations simplify the model into curves, discretize curves, and then optimize the inner stress and moment equilibriums through evolutionary algorithms (Figure 11a).

To reduce the calculation time, we created a database that contains a group of precalculated curves that fulfill various boundary conditions (Figure 11b). To simulate a model, we first simplified the model into a combination of basic curves, depending on whether it had the spore actuators on top of the substrate material or not. We then investigated the database
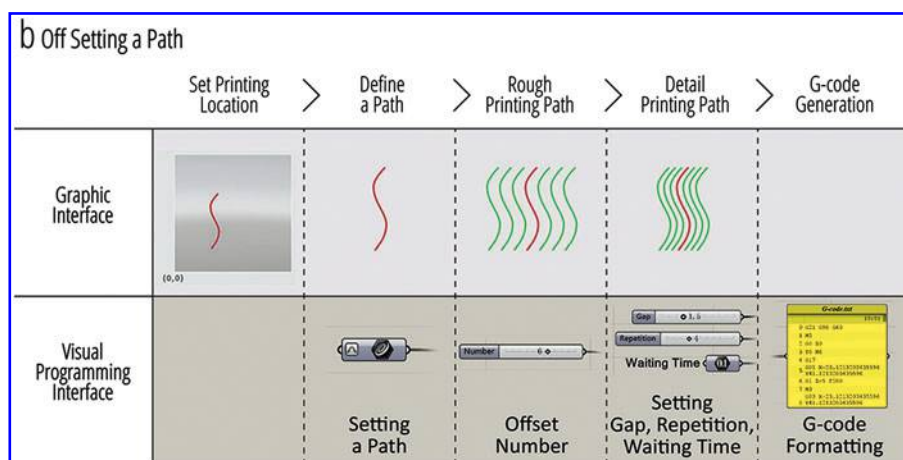
FIG. 8. Graphic user interface for offsetting a 1D line. Color images available online at www.liebertpub.com/3dp

and approximated the shape of each basic curve via the interpolation of the existing data. These curves were then connected to achieve a rough shape of our model. To refine the rough simulation, we applied the same evolutionary computing method to the entire model within a reduced range of possible solutions.

Customized G-code

We assigned some extended G-code with new functions to gain additional controllability over the dispensing module and the agitation module (Table 1).

Operation

Procedure

The operations on the printing machine include design and G-code generation, loading spore solutions, mounting the material substrate, tuning the agitator and ventilating fans, and sending G-code and running the machine. A thorough cleaning process with isopropyl alcohol is required after printing (Figure 12).

Calculating the flow rate

We can control the spore layer thickness via a combination of the flow rate and the machine feedrate. To reach a certain curvature under certain relative humidity (RH) conditions,

we can refer to Figure 12 and interpolate the suggested spore layer thickness t. To reach t, it takes time T:

$$t \times S = v \times T \tag{1}$$

where S is a certain surface area and v is the machine flow rate.

To allow the deposition to cover the entire surface area S,

$$f \times T \times w = S/z \tag{2}$$

where f is the machine feed rate, w is the width of the droplet coverage, and z is the stepover of the CNC machine.

Machine feed rate v can be calculated based on Equations (1) and (2):

$$f = v/(t \times z \times w) \tag{3}$$

The above calculation supported our design practices in terms of the following step: via printing density, orientation, and resolution, we were able to control the material properties of the biohybrid film, as well as the transformational behaviors of such films.

Printing Primitives

With biofilms as basic building blocks, we designed responsive structures and transformations that could be
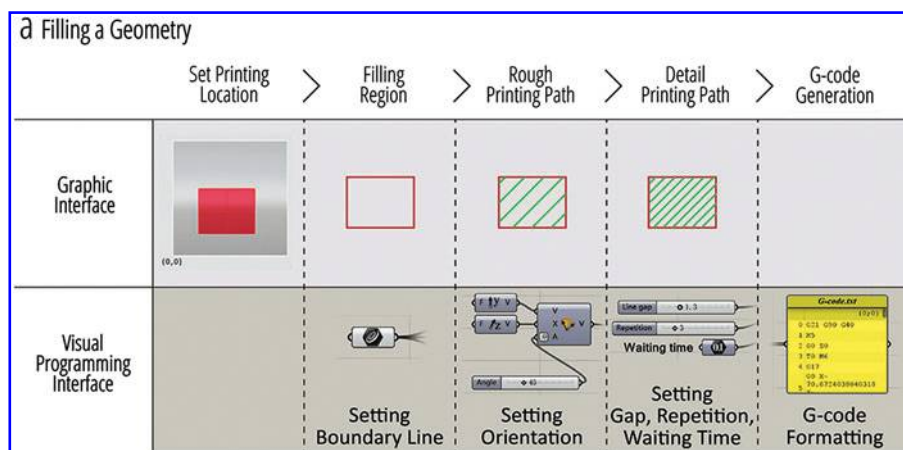

FIG. 9. Graphic user interface for filling a 2D outline. Color images available online at www.liebertpub.com/3dp
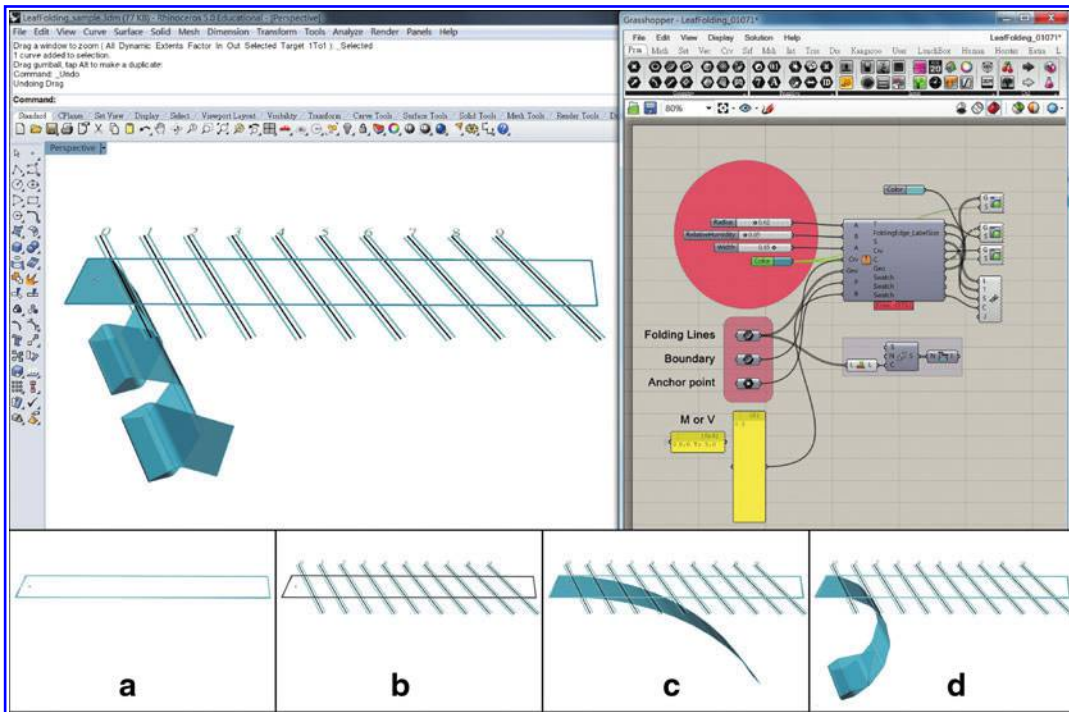
FIG. 10.   Design and simulation platform. **(a)** Defining substrate. **(b)** Defining actuators. **(c)** Simulating in high RH. **(d)** Simulating in low RH. Color images available online at www.liebertpub.com/3dp

referenced when attempting to achieve a certain shape change in the design of interactive systems.

Transformation design is based on two bending primitives (Figure 13): curved bending is used for more organic transformation, whereas angular bending is used for more geometric transformation. To achieve a curving transformation, spores were applied across the entire substrate; for an angular transformation, spores were applied in lines on top of the substrate. In the latter case, a stiffer material can be attached to substrate regions without spore actuators, to stabilize the structure and enhance the effect of a sharp fold.

By combining the bending primitives across different dimensions, we can create a variety of responsive transformations, including lD linear transformation, 2D surface expansion and contraction, 2.5D texture change, and 3D folding (Table 2).

Applications

Transforming plants

On the basis of the principle of programming transformation through orientation spore deposition, we printed leaves with different shapes. Spore actuators followed the vein structure of certain leaves, which effected the biomimetic transformation of leaves that resembled real natural organisms (Figure 14). Considering that many natural leaves transform because of the gain and loss of water inside their veins, here, spore actuators swelled and shrunk to create a similar effect.

With a closed control loop, artificial plants that respond to various stimuli can be designed as educational toys. To concurrently mimic a natural flower changing shapes and color, we mixed thermochromic paint into liquid latex to produce our own color-changing film substrate. A flower bouquet was
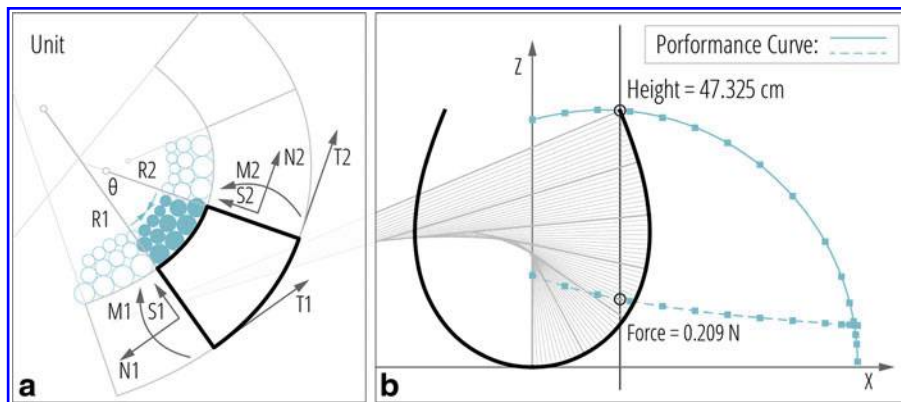

FIG. 11.   **(a)** Design and simulation platform. **(b)** The analysis of force and moment in a single unit with the spore stress and geometric constraint. **(c)** The shape of basic curve generated by interpolating data from the database. Color images available online at www.liebertpub.com/3dp

TABLE 1. g-CODE WITH CUSTOMIZED FUNCTIONS

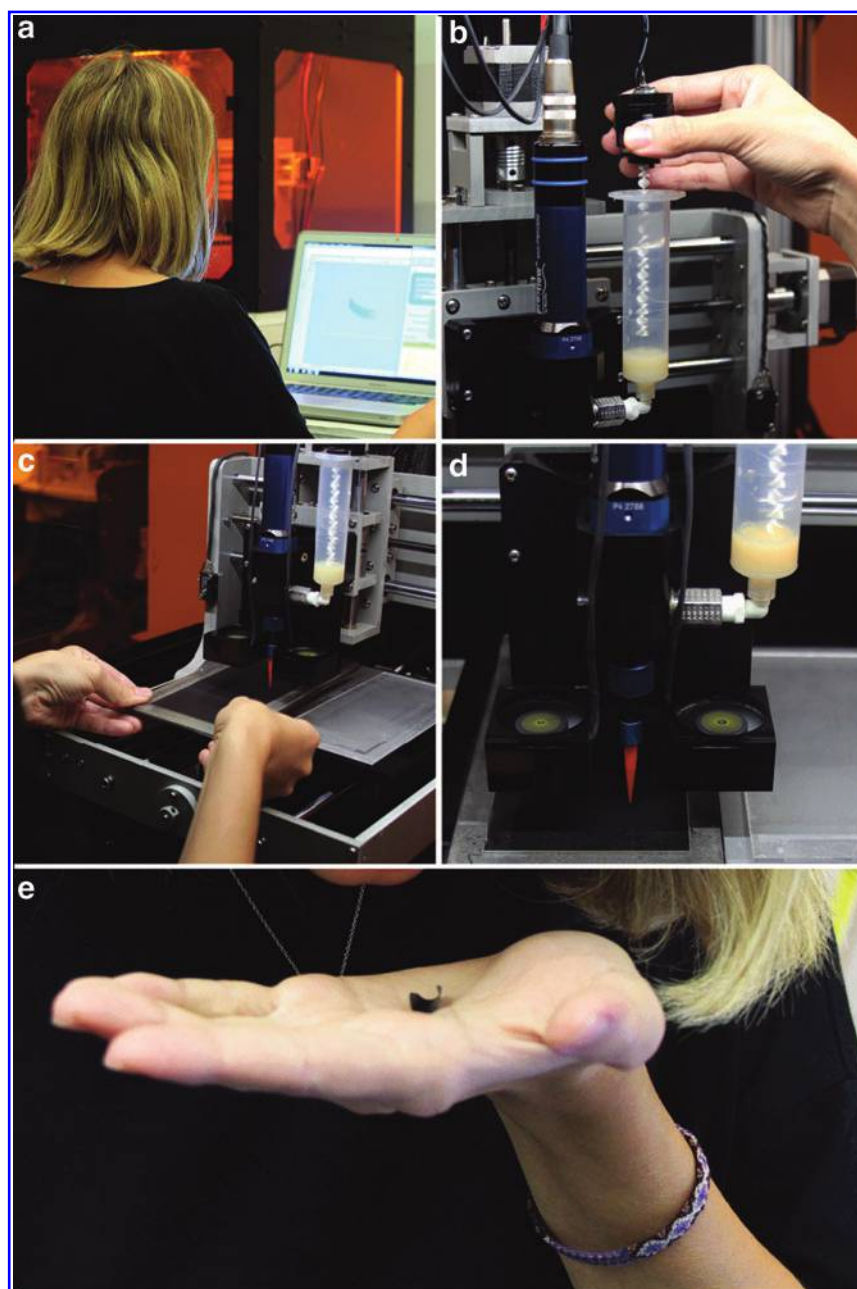| g-Code	Old function	New function	Control circuit
M3	Turn spindle clockwise	Turn on the trigger for the dispenser module	Output high signal to dispenser control board
M5	Stop spindle	Turn off the trigger for the dispenser module	Output low signal to dispenser control board
M42 P27 S255	Second fan control	Switch on the agitator module	Output high signal to the agitator motor control circuit
M42 P27 S0	Second fan control	Switch off the agitator module	Output low signal to the agitator motor control circuit
M140 S255	Heating bed control	Turn on the fan	Output high signal to the fan control circuit
M140 S0	Heating bed control	Turn off the fan	Output low signal to the fan control circuit

FIG. 12.   **(a)** Load the design in the software platform. **(b)** Activate the stirring component. **(c)** Place the substrate. **(d)** Printing. **(e)** Testing the transformation with breath. Color images available online at www.liebertpub.com/3dp
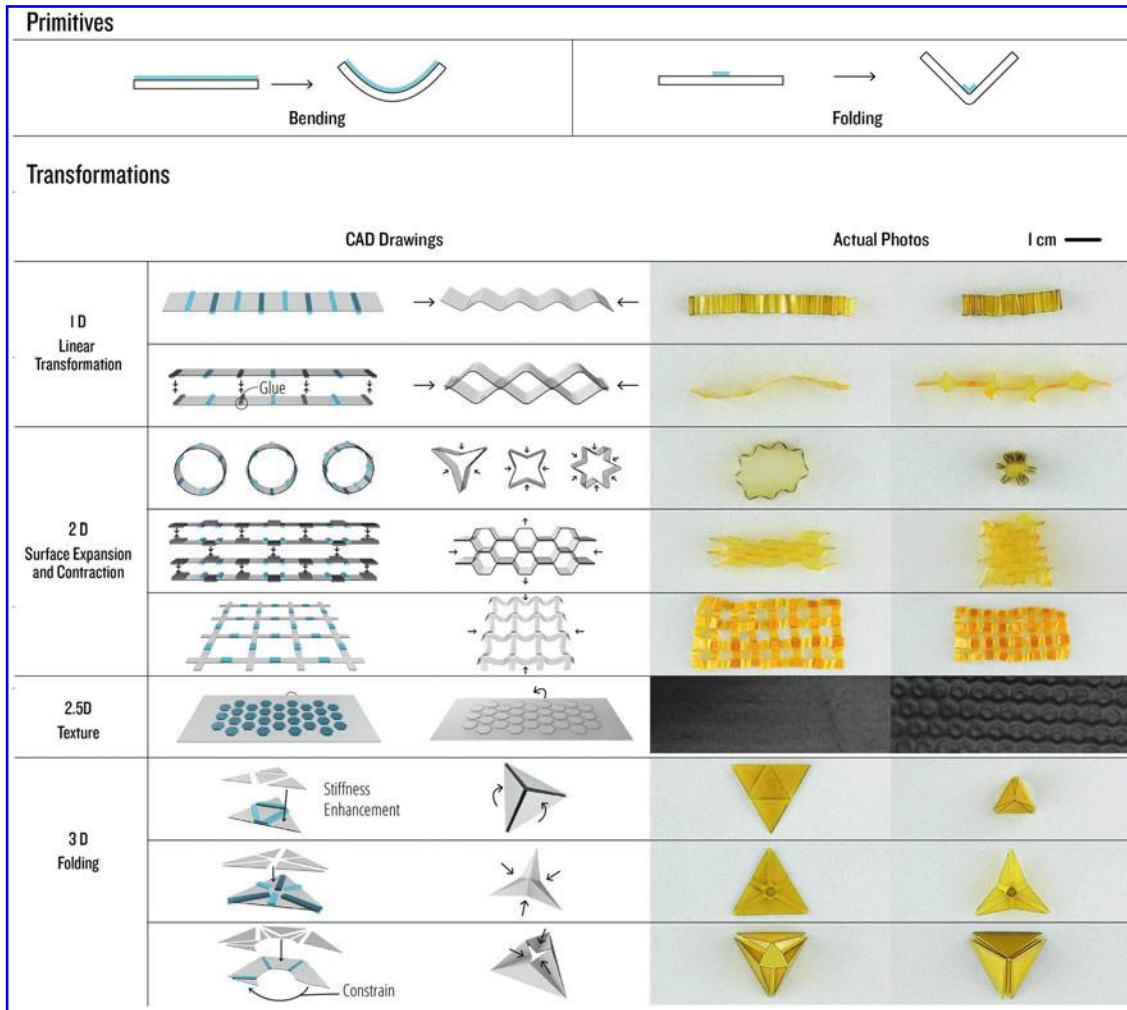
FIG. 13.   Design of responsive structures. Bending and folding primitives can be translated into 1D linear transformation, 2D surface expansion and contraction, 2.5D texture change, and 3D folding. Color images available online at www.liebertpub.com/3dp

designed to transform both shape and color (Figure 15). When we sprayed water on the bouquet, it changed from being curled and umber to expanding and red. The control logic here is that the heating circuit below the plate is always on until an equipped humidity sensor detects a water spray.

Living teabag

We applied the transformable leaves to the design of ''living'' tea bags. A leaf at the top of the tea bag is initially curled up. After pouring hot water into a teacup, the curled leaf slowly unwraps to indicate that the tea bag is fully soaked in water. Once the tea is ready and the tea bag is pulled out of the cup, the leaf will curl up again to indicate the end of its life (Figure 16). The unwrapping can be triggered by either steam coming from the hot water, or by moisture drawn up by capillary force from the tea bag. Since we can control the length and timing of capillary movement, the unwrapping leaf can more precisely indicate when the tea is ready.

Responsive Lamp

Using one of the 3D folding primitives mentioned earlier, we were able to design a responsive lamp that closes its lampshade when it is turned off and opens up to leak light and

create lighting patterns when it is turned on (Figure 17). Using this example, we intend to demonstrate the fabrication techniques that we suggested were easily extendable to more complex systems with more transforming units. All of the actuators on this lamp were fabricated within 12 hours by two experienced fabricators.

Figure 14d–f depicts another approach for interacting with artificial plants. This leaf mimics the movement of Mimosa, which closes when people touch it. Capacitive sensing uses the same circuitry for heating. Two relay switches control the embedded conductive traces to be connected to either a capacitive sensing board or a direct voltage source for heating.

Living Wall

This application focuses on the transformation of permeability in response to environmental stimuli. Living surfaces can be adapted to different use cases; for example, a living window that responds to humidity changes. The window curls up to let sunlight through when it is sunny and closes off when it is cloudy or rainy (Figure 18). It can also be used as a shower curtain that will automatically close off when the shower is open. Finally, it can be a living wall that responds to
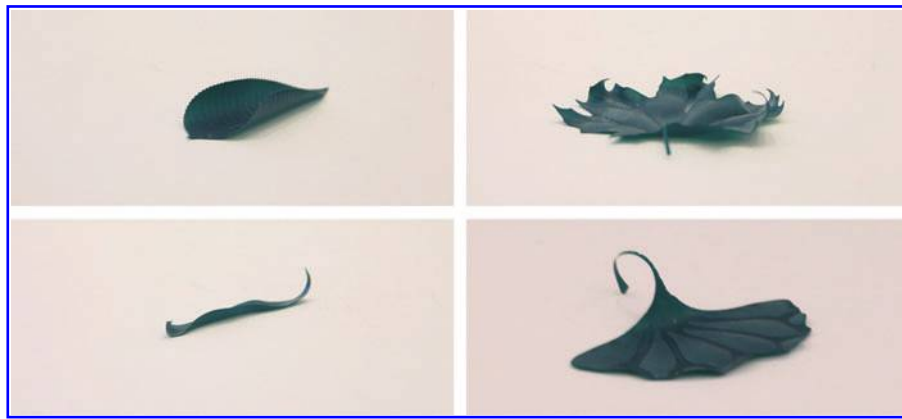
FIG. 14.   Spore actuators follow the vein structures of leaves to create biomimetic transformation. Color images available online at www.liebertpub.com/3dp

FIG. 15.   Artificial flower bouquet that changes both color and form. **(a)** Bouquet at low RH. **(b)** Spraying water. **(c)** Changing color and shape. Color images available online at www.liebertpub.com/3dp

FIG. 16.   A teabag with an artificial transformable leaf as an indicator. Color images available online at www.liebertpub.com/3dp

FIG. 17.   Transformable lampshade. The flaps open up as the temperature rises, causing RH to drop. Color images available online at www.liebertpub.com/3dp

human exhalation and exposes different patterns to create interactive experiences.

Challenges and Future Work

Control axis and CNC moving speed

During our study, we learned that printing on both sides of a thin film to create composite structures is feasible. For improvement, we would like to build a rotatory printing platform that can flip the printing bed upside down; we would also like to increase the speed of the printing by replacing the current servo motors with faster and steadier ones.

Multimaterial printing with customized central dispenser

We currently use an off-the-shelf central dispenser. It is very precise, but costly and limited to one print head. For the next step in our research, we would like to develop our own open source progressive cavity pump-based dispensing system. We would like to have multiple containers that can print more than one material at the same time. Doing so will create opportunities for 3D printing with supporting structures and composite material printing with embedded functions.
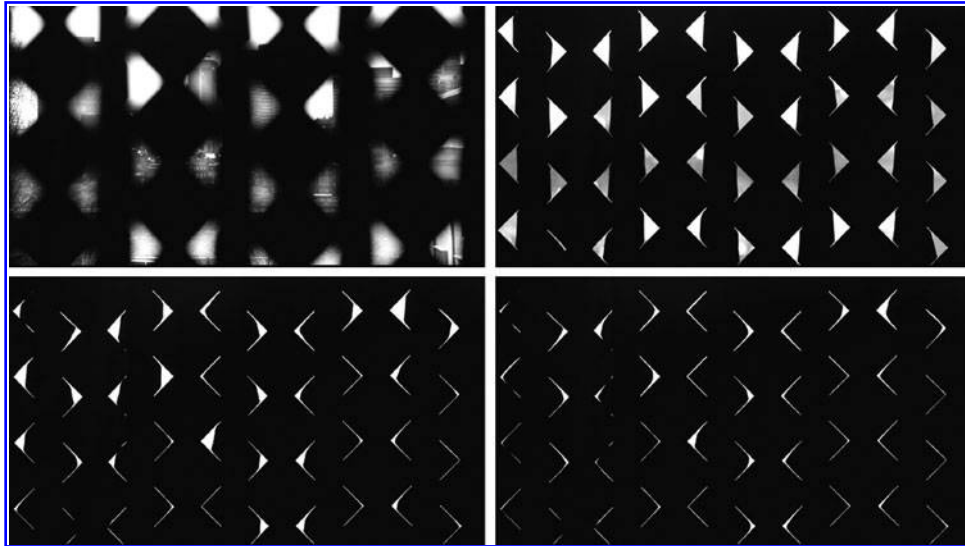
FIG. 18.   A living wall that changes its permeability based on the environmental relative humidity change.

Open source hardware and software

Although this article details all the design principles of building a liquid deposition-based cell printer, we would like to construct a wiki page in which we can share our source code for the software design and simulation platform, and in which we can also document our machine design process. Community involvement and support is a powerful approach for improving such a system through practice and comments.

Conclusions

In this article, we described a digital fabrication pipeline for depositing a suspension of *B. subtilis* endospores, a hygromorphic natural material, on thin substrates. The proposed hardware and software platform enables designers to create biohybrid materials that transform in response to relative changes in environmental humidity. We also presented different applications based on this specific material and printing system. In the future, we hope to further develop this platform for more versatile printing, more functional material printing, as well as more sophisticated 3D structures generation.

Author Disclosure Statement

No competing financial interests exist.

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