3D PRINTING AND ADDITIVE MANUFACTURING
Volume 3, Number 4, 2016

DOI: 10.1089/3dp.2016.0040

ORIGINAL ARTICLE

Spatial Control of Bacteria Using Screen Printing

Soonhee Moon,[1,*] Ian L. Fritz,[1,*] Zakary S. Singer,[1,*] and Tal Danino[1–3]

Abstract

Synthetic biology has led to advances in both our understanding and engineering of genetic circuits that affect spatial and temporal behaviors in living cells. A growing array of native and synthetic circuits such as oscillators, pattern generators, and cell–cell communication systems has been studied, which exhibit spatiotemporal properties. To better understand the design principles of these genetic circuits, there is a need for versatile and precise methods for patterning cell populations in various configurations. In this study, we develop a screen printing methodology to pattern bacteria on agar, glass, and paper surfaces. Initially, we tested three biocompatible resuspension media with appropriate rheological properties for screen printing. Using microscopy, we characterized the resolution and bleed of bacteria screen prints on agar and glass surfaces, obtaining resolutions as low as 188 μm. Next, we engineered bacterial strains producing visible chromoproteins analogous to the cyan, magenta, and yellow subtractive color system for the creation of multicolored bacteria images. Using this system, we printed distinct populations in overlapping or interlocking designs on both paper and agar substrates. These proof-of-principle experiments demonstrated how the screen printing method could be used to study microbial community interactions and pattern formation of biofilms at submillimeter length scales. Overall, our approach allows for rapid and precise prototyping of patterned bacteria species that will be useful in the understanding and engineering of spatiotemporal behaviors in microbial communities.

Keywords: bioprinting, medical applications, additive manufacturing processes, design

Introduction

The rapidly developing field of synthetic biology has opened new channels for understanding and designing microbial behaviors. These behaviors, often controlled by core genetic modules consisting of positive and negative feedbacks, can be genetically modified to probe their natural function or co-opted for novel biotechnological applications. In the context of applications, genetic circuits have been rewired to dynamically sense chemical pollutants,[1,2] cancer,[3–5] pathogenic microbes,[6,7] and multiple chemical inputs using logic gates.[8] A variety of genetic circuitry to spatially control bacteria has been developed,[9–12] typically using quorum sensing to allow for information exchange across a population through a small diffusible molecule.

As applications of engineered genetic circuitry in bacteria continue to develop, a natural question arises as to how these systems will be utilized in the field. One promising direction for the usage of genetic circuitry is in the context of paper-based sensors, which are a low-cost, disposable, and portable technology that can be used for a wide variety of purposes.[13–15] Although most paper-based sensors have used detection elements consisting mainly of enzymes and antibodies, little effort has been devoted to whole-cell biosensor approaches in the context of paper-based sensors. To manufacture these, a consistent method for printing bacteria or other cells onto paper substrates would need to be developed. Such a method would ideally allow for simple and consistent printing, rapid prototyping, and scalability.

Over the last few decades, several technologies to control the patterning of cells at various scales have been developed, including bioprinting, microfabrication, and photopatterning.[16–21] Each of these techniques has unique advantages for the specific biological question studied and can be parsed into indirect cell

[1]Department of Biomedical Engineering, Columbia University, New York, New York.
[2]Data Science Institute, Columbia University, New York, New York.
[3]Herbert Irving Comprehensive Cancer Center, Columbia University, New York, New York.
*These authors contributed equally to this work.

Opposite page: Two strains of *E. coli* expressing eforRed and amilGFP fluorescent proteins screen printed onto agar Petri dishes. *Photo credit:* Soonhee Moon.

patterning by substrate control or direct cell patterning. Commercial inkjet printers or repurposed home printers have successfully printed both macromolecules that control cellular growth and fate, and directly printed bacteria, neurons, and other mammalian cell types.[22–25] Inkjet printing of cells can lead to high precision ($\sim 20\,\mu$m resolution); however, clogging of print heads can lead to inconsistencies in printing and high maintenance. Microfabrication techniques are often able to pattern both cells and substrates even more precisely, although large-scale patterning can be challenging, and techniques often require specialized equipment or facilities. Photopatterning approaches have demonstrated control of substrates in 2D/3D and hence growth and division, although mainly in the context of mammalian cells.

Screen printing is a type of stenciling technique that is often utilized for its versatility, rapid prototyping, and ease of manufacturing. Unlike stenciling, which requires ''bridges'' connecting the floating parts of an image, screen printing evolved as a solution for producing more complex images. The technique uses a mesh to transfer ink onto a surface, except in areas impermeable to ink by a blocking stencil. Ink is traditionally applied with a squeegee moved across the screen to fill the open areas with ink. By repeating this process, multiple layers of ink can be applied in succession, allowing for control over layer thickness. In addition, several colors and types of ink can be applied in distinct regions with multiple screens. Because of these features, screen printing has been widely used for commercial printing with inks, conductive electrodes, and enzymatic biosensors, however, less attention has been devoted to screen printing living cells.[26–28]

To screen print effectively, the rheological characteristics of ink are important to optimize. Screen printing inks have high viscosity and show plastic or pseudoplastic properties (similar to ketchup), allowing them to flow smoothly after being subject to shear. Furthermore, they often display thixotropy, continuing to decrease in viscosity in a time-dependent manner even at a fixed shear rate, which is important for proper ink behavior.[29] In a normal screen printing process, ink molecules are mixed into a medium with desired rheological properties, which is then used to make prints. In our application, the bacteria will substitute as the ''ink,'' and are adjusted to have similar properties to a screen printing ink by use of a resuspension medium. An additional consideration is the biocompatibility of the screen printing medium to allow for microbial growth. In this study, we will develop several aspects of screen printing bacteria onto different surfaces (Fig. 1).

Materials and Methods

Media

Solutions of 4% w/v carboxymethyl cellulose (CMC; CK Products, USA), 1.2% w/v Xanthan gum (XG; Now Foods, USA), or 49.7% w/v gum arabic (GA; CK Products) were created by stirring and heating to 50°C in water until fully dissolved. CMC was nearly optically clear, whereas GA and XG were opaque. The screen-printed agar dishes composed $0.25\times$ Lennox lysogeny broth (LB; Molecular Biologicals International, Inc., USA; 0.25% tryptone, 0.25% NaCl, 0.125% yeast extract) and solidified with 1.5% agar, supplemented with necessary antibiotics (ampicillin 100 μg/mL) unless otherwise noted. Pattern-forming bacteria were grown on agar dishes composing 0.5% NaCl, 0.5% K_2HPO_4, and 0.2% peptone, and solidified with either 1.5% or 0.9% agar. 12×12 cm (Gosselin, France) and 24.5×24.5 cm Petri dishes (Corning, USA) were used for screen printing and completely filled such that the screens would be able to contact the agar. For black ink and silk screen medium, we used $\sim$25 mL of either water-based AquaBrite textile colors (Holden's Screen Supply, USA) or a water-based Golden Silkscreen medium (Golden Artist Colors, Inc., USA).

Screen printing

The amount of CMC or XG necessary to screen print was dependent on individual screen mesh sizes, screen designs, and desired density of bacteria cultures. Screens were purchased from Holden's Screen Supply. Screen sizes were 18×20 inches, 21×27 inches, and 11×14 inches, at 305 mesh count. Generally, 12.5 mL of medium were sufficient to screen print per agar dish. The solution was applied to the screen and pulled through using a 10-inch squeegee. One pass was optimal to prevent bleeding and smearing, but two were conducted if a sufficient amount of bacteria was not transferred during the first pass. To increase contact between plates and screens, the plates were elevated, such that the screen was pulled down around the edges of the plates.

Strains

Plasmids containing genes for *eforRed*, *amilGFP*, and *cjBlue* were chemically synthesized (Genscript, USA), containing the pTac promoter driving a gene of interest. The promoter sequence and ribosome binding site (RBS) used are as follows: 5′-GAGCTGTTGACAATTAATCATCGGCTC GTATAATGTGTGGAATTGTGAGCGGATAACAATTT

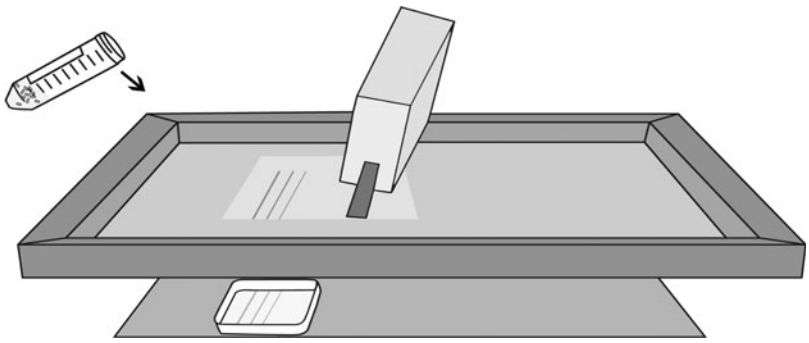

FIG. 1. Screen printing bacteria. Bacteria are resuspended in a biocompatible, viscous medium. The mixture is placed along the edge of the screen and is applied by moving the squeegee horizontally across the screen. The pattern in the screen is then transferred onto an agar dish (or other surface) placed underneath the screen and allowed to grow and develop.

CACACAGGAAACAGAATTCT-3′. All plasmids were synthesized in a pBluescript II KS + vector and were transformed into a Mach1 strain of *Escherichia coli* (Life Technologies) and plated on LB at 37°C. For preparation of bacteria for screen printing, cultures were grown at 37°C with shaking overnight. One hundred sixty microliters of culture were then transferred to 25 mL desired screening medium. For screen prints of overlapping circles, 500 mL of culture were grown at 37°C with shaking for 2 days. The culture was then centrifuged for 10 min at 10,000 g and 20°C. Resuspension and centrifugation were performed as noted to concentrate bacteria. *Paenibacillus dendritiformis T* and *P. dendritiformis C* were kindly provided by Eshel Ben-Jacob and grown at 37°C for all cultures. Staining was performed with 40% methanol, 20% acetic acid, 19.9% dH2O, and 0.1% Coomassie blue (or 0.1% Coomassie blue and 0.1% methyl red solution). Ten microliters of solution were added to the dish and left to sit for 5 min, after which it was decanted. Ten microliters of 40% methanol, 20% acetic acid, and 20% dH2O were then used to destain the plate for 10 min, or more as needed.

Imaging

75 × 25 mm glass slides (VWR, USA) were used for imaging bacteria on Nikon TI-E with Perfect Focus. Images of all plates were taken using a Canon EOS 5D Mark II camera. Plates were placed on a light box, with the camera mounted on a tripod above them.

Resolution measurements and statistics

Measurements of the positive resolution and bleed were conducted by measuring the width of a given screen-printed line using a Nikon TI-E microscope with a CFI Plan Fluor DL 4X objective. Five width measurements were then taken per line, and the mean and standard deviation were calculated.

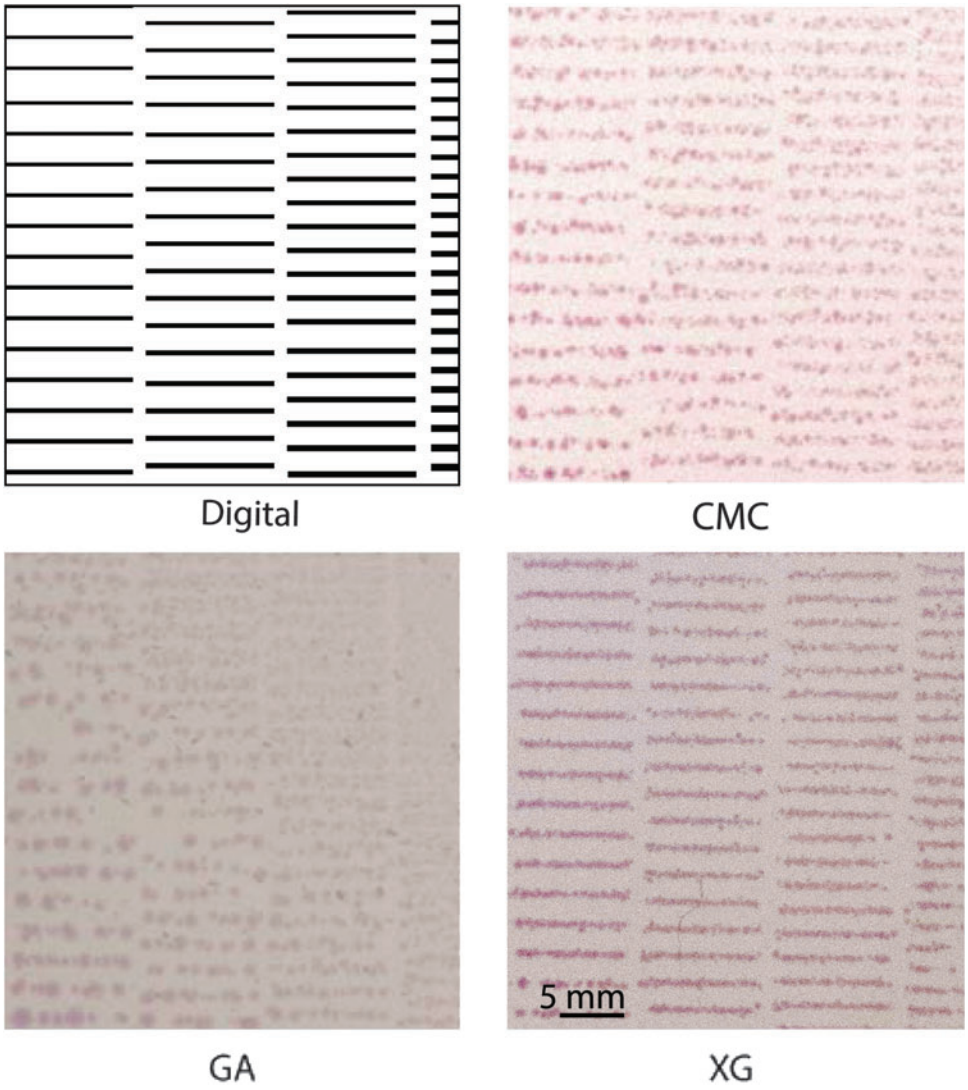

FIG. 2. Bacterial growth following screen printing. *Top left:* A portion of the screen design containing decreasing line spacing from *left* to *right*, and increasing line thickness from *top* to *bottom. Other panels:* Results of *eforRed* bacteria resuspended in three mediums, screen printed onto agar dishes, and grown for 2 days at 37°C for color to be visible. The three mediums are XG, GA, and CMC. Photos were taken with a digital camera illuminated by an LED light panel placed underneath the agar dishes. Scale bars apply to all images. CMC, carboxymethyl cellulose; GA, gum arabic; XG, Xanthan gum.

Results

Screen patterning mediums for bacteria

To allow for simple visualization of bacteria during screen printing, we engineered a strain of bacteria to express *eforRed*, a red chromoprotein, under control of a constitutive pTac promoter. This strain also contains an antibiotic resistance marker, ampicillin, which prevents contamination from outside bacteria during screen printing. We initially mixed *eforRed* bacteria cultures into a commercially available silk screen medium. To screen print the bacterial mixture onto an agar dish, 12 cm Petri dishes were filled completely with a nutrient agar mixture. A 21×27 inch screen was clamped on a table, and then aligned with the agar dish underneath it. Approximately 12.5 mL of the bacterial mixture were placed on the screen and applied to the agar dish using a squeegee (Fig. 1). The agar dish was then removed and placed in a 37°C incubator to allow for bacterial growth.

After screen printing directly onto a Petri dish as described above, we surprisingly observed no bacterial growth. A control streak of bacteria without the silk screen medium plated on an agar dish grew and expressed *eforRed*; however, a control streak of bacteria with the silk screen medium plated on an agar dish did not grow (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/3dp). We additionally tried two other fluorescent bacterial strains and observed no bacterial growth as well. Based on these results, we concluded that there was some toxic element present in the silk screen medium that prevented bacterial growth. The silk screen medium's material data safety sheet did not explicitly describe in detail its composition, making it difficult to determine which ingredients in the formulation may be inhibiting bacterial growth.

We therefore sought to determine an appropriate medium that would allow for screen printing bacteria. Such a medium would have both appropriate viscoelastic and biocompatible properties. To narrow our search range, we chose food-grade thickening agents that were likely to be biocompatible and not interfere with bacterial growth, and also displayed thixotropic properties for accurate screen printing. We characterized three possible chemical thickeners to use as resuspension mediums: (1) XG, an extracellular polysaccharide of the bacterium *Xanthomonas campestris*,[30] (2) GA, a natural gum made from the hardened sap of the acacia tree,[31] and (3) CMC, a derivative of cellulose found in plants.[32] Each solution was formulated to a concentration that was qualitatively similar in viscosity to the commercially purchased silk screen medium.

Resolution and morphologies of bacteria screen prints

Having identified possible screen printing mediums, we explored and characterized bacterial growth after screen printing onto agar surfaces. Using a screen containing a design with varied line thicknesses and spacings (Fig. 2A), we screen printed *eforRed* bacteria mixed with XG, GA, and CMC onto

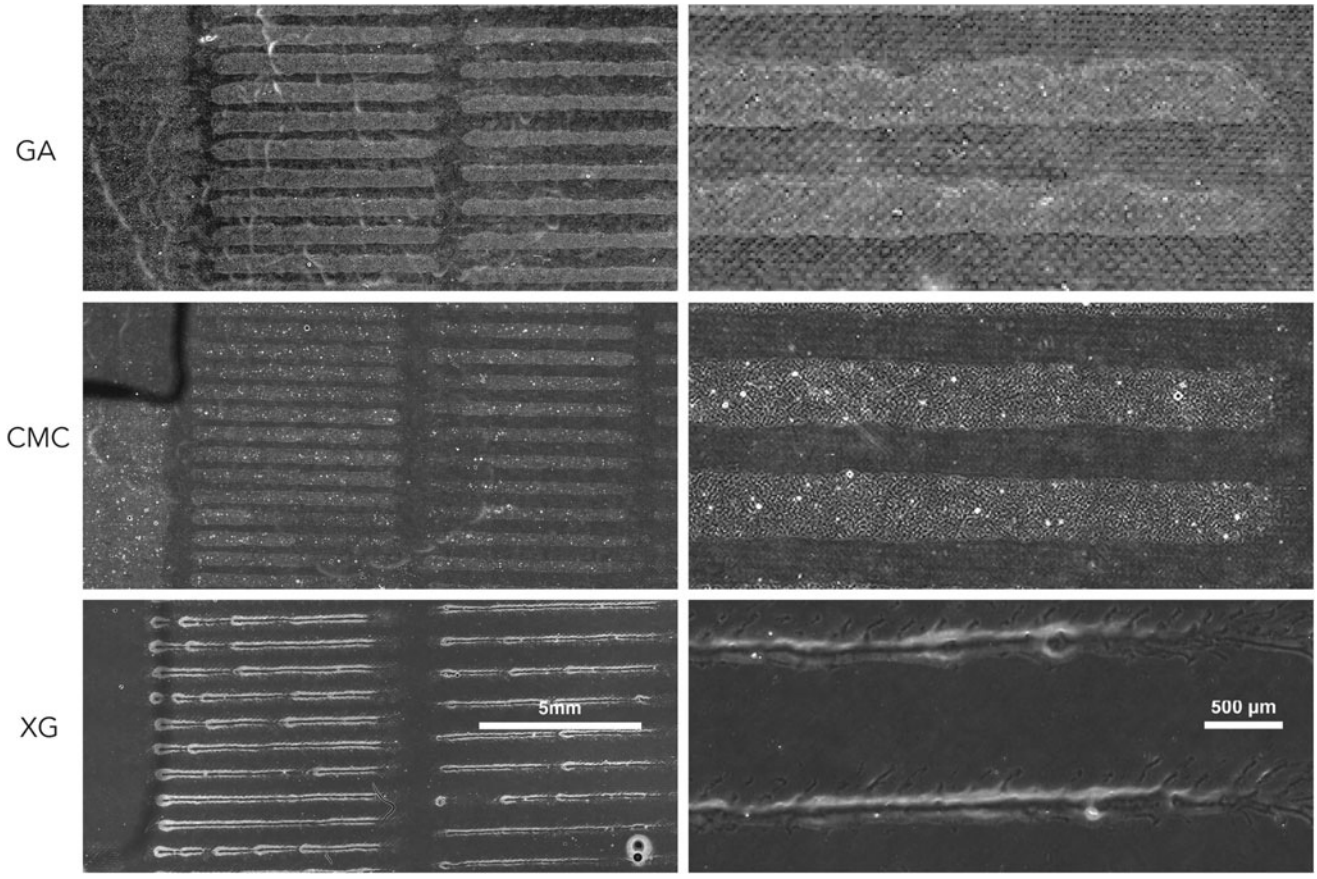

FIG. 3. Microscopic characterization of bacteria screen printing on agar. Images taken at 4× magnification of XG, GA, and CMC *eforRed* bacteria mixtures immediately after screen printing on agar, allowing for quantification of positive resolution, bleed, and morphological features. Scale bars shown apply to all images in a column.

Table 1. Measurements of Positive Resolution and Bleed for Screen Printing Mediums

	GA		*XG*		*CMC*	
	Positive Resolution	*Bleed*	*Positive Resolution*	*Bleed*	*Positive Resolution*	*Bleed*
Glass	251 ± 24	171 ± 24	207 ± 33	67 ± 33	412 ± 28	332 ± 28
Agar	388 ± 26	248 ± 26	188 ± 34	8 ± 34	323 ± 16	203 ± 16

CMC, carboxymethyl cellulose; GA, gum arabic; XG, Xanthan gum.

agar dishes and allowed them to grow in an incubator at 37°C for 2 days (Fig. 2B–D). The growth of the bacterial colonies produced the *eforRed* protein and allowed for simple visualization with a digital camera. We observed that the features of the three mediums seemed to differ. While the XG mixture seemed to produce finer lines with tighter intercolony spacing, CMC and GA produced colonies that were sparser, with GA colonies often inconsistently printed. It was unclear whether these apparent features were a result of growth of bacteria after screen printing or due to the differences in the screen printing process itself. Thus, to investigate these effects, we turned to microscopy for further characterization.

We used the same screen with lines of varying thicknesses and spacing and screen-printed bacteria in GA, XG, or CMC onto either glass or agar, and imaged them under a microscope at 4× magnification (Fig. 3). The resulting images demonstrated that the combination of medium and surface yielded distinct line morphologies and positive resolutions (Table 1). We defined positive resolution by finding the first set of three contiguous lines in a given image and measuring width of thinnest of these lines at multiple points along the line (Materials and Methods). Immediately after printing on agar (Fig. 3), XG appeared to coalesce and form narrower lines than the other two mediums (188 ± 34 μm), while leaving spines on either side of the line itself (the spines themselves were excluded from the measurement of this thickness). In contrast, GA and CMC produced thicker lines of 388 ± 26 and 323 ± 16 μm, respectively. Performing analogous experiments on glass (Supplementary Fig. S2), CMC yielded lines of positive resolution at 412 ± 28 μm, while XG and GA formed narrower lines at 251 ± 24 and 207 ± 33 μm, respectively.

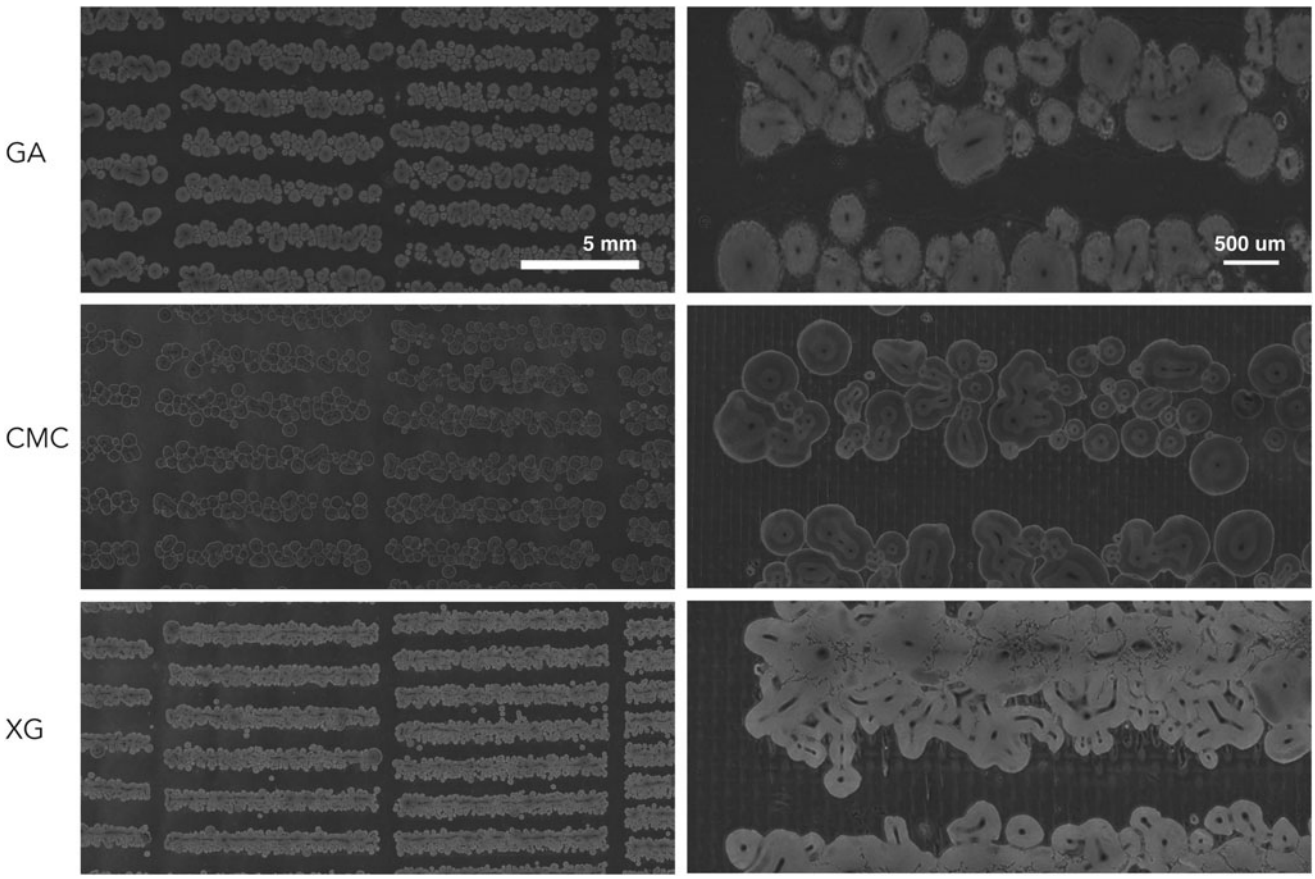

FIG. 4. Microscopic characterization of bacteria and colony morphology after screen printing. Screen-printed *eforRed* bacteria in XG, GA, and CMC on an agar dish, grown for 2 days at 37°C, and visualized by microscopy. Colony morphology differs between GA, CMC, and XG, although no notable changes in growth rate were observed. XG produces the highest density growth and finest lines, in particular along the midline of the lines, with CMC and GA producing sparser colonies. Scale bars shown apply to all images in a column.

In further contrast with agar, CMC and GA on glass formed prominent spines flanking the lines.

In addition to positive resolution, we also computed the ''bleed'' across all combinations of medium and surfaces (Table 1). This was defined as the difference between the positive resolution and the actual thickness of the intended line according to the digital image. XG yielded a remarkably low bleed on both agar and glass, and significantly less bleed than either GA or CMC on both surfaces. As a point of comparison, we screen printed commercially available black silkscreen ink on glass and obtained line thicknesses of 429 ± 25 μm, with a bleed of 349 ± 25 μm.

Finally, we asked whether bacteria were still viable after screen printing. Indeed, all mediums permitted bacterial growth, although with distinct colony morphologies (Fig. 4): GA appeared to produce a wide array of colony sizes with nonuniform edges; in XG, bacterial growth appeared denser and homogenous throughout the line; and CMC produced discrete colonies of homogenous sizes, but with more similar properties to normal colonies observed without a resuspension medium. Together, these distinct morphological properties suggest that the choice of surface and medium may vary by application, but XG may enable the highest printed resolution.

Complex configurations of screen-printed bacteria

Communities of bacteria are known to interact with one another, either competing and inhibiting each other's growth or engaging in cooperative behaviors.[33–35] We sought to determine if our screen printing approach could be used to incorporate multiple bacteria populations in more complex designs. To do this, we engineered additional bacteria producing yellow (*amilGFP*) and blue (*cjBlue*) proteins under the pTac promoter to allow for visualization of distinct populations. Along with our previous red (*eforRed*) protein, this created a distinct cyan, magenta, and yellow color system that could produce a broad color spectrum in bacterial mixtures. Using a ''venn diagram'' design of overlapping colors, we screen printed a dense bacterial mixture in CMC onto paper with three successive screen prints of *amilGFP*, *eforRed*, and *cjBlue* bacteria, respectively (Fig. 5A). The resulting print demonstrated color mixing similar to the combination of traditional pigments. This type of screen-printed design could be representative of what interacting bacteria biosensors on paper would look like.

We also tested if screen printing could produce more complex images and configurations with shared interfaces. We first printed and grew single populations in more complex ''cube'' designs using *amilGFP* bacteria with CMC, showing the versatility of the screen printing approach (Fig. 5B). Next, we printed *amilGFP* and then *eforRed* bacteria mixtures on an agar dish in the design presented in Figure 5C. The pattern was well maintained after 2 days of growth and produced the yellow and red pigments. We additionally imaged this pattern under blue-light transilluminator (on a standard gel box with and orange emission screen) in Figure 5D to provide more contrast. These experiments provided a proof-of-principle system that could be utilized to investigate interactions and competitions between juxtaposed strains in future studies.

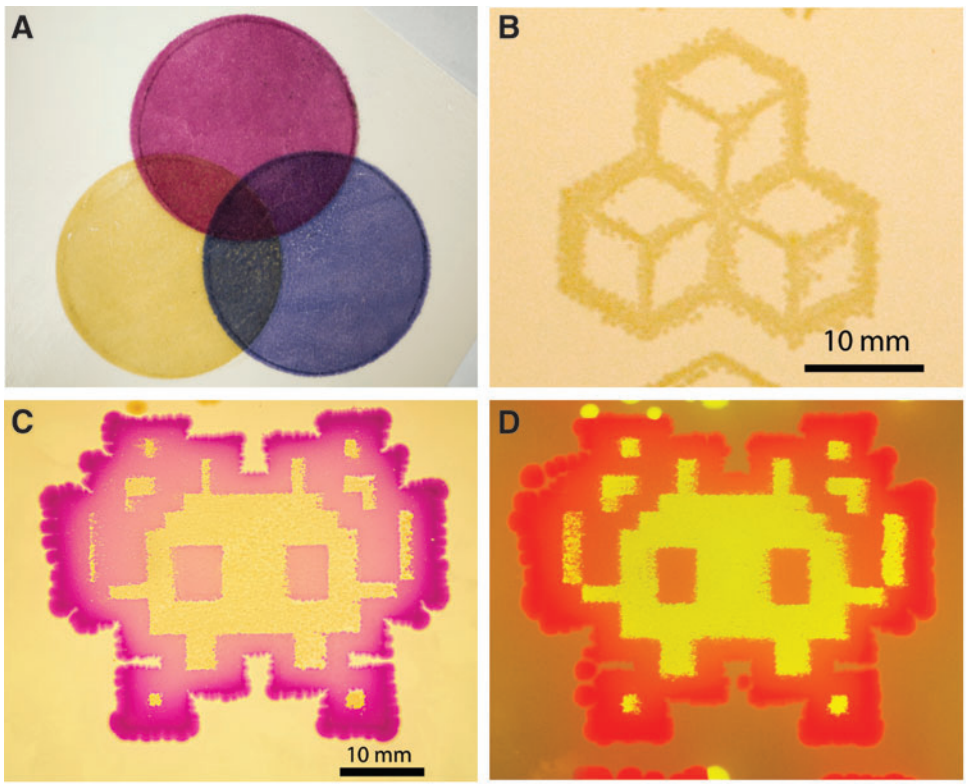

FIG. 5. Complexity and multipopulation screen printing of bacteria on surfaces. **(A)** Successive screen prints of *amilGFP*, *eforRed*, and *cjBlue* bacteria on paper demonstrating color mixing of bacteria. **(B)** *amilGFP* screen printed in cube shape and grown on agar at 37°C for 2 days. **(C, D)** Successive screen prints of *amilGFP* and *eforRed* bacteria in an interlocking configuration grown for 2 days on agar. Scale bars shown apply to images on each row.

Biofilm pattern formation using screen printing

Some bacteria form striking, fractal-like patterns that resemble nonliving systems, but as they grow spontaneously change into a new state that results in a distinct morphology.[36–40] One potential question in studying these biofilms is what determines the sudden transitions and how far back the spatial history of the pattern influences the biofilm's next transition. An approach to study this question is to pattern bacteria into their natural fractal shapes as the initial conditions and vary the conditions at which bacteria were prepared (e.g., different bacteria subtypes, densities, and nutrient conditions). To demonstrate how screen printing could be used for this application, we grew *P. dendritiformis T* bacteria[40] under pattern-forming conditions until they reached the shape observed in Figure 6A. We stained and imaged the resulting pattern and created a screen with an identical shape. The feature sizes of the bacteria pattern were on the order of a millimeter or less, appropriate for the resolution we can obtain when screen printing. We then patterned *amilGFP* bacteria (Fig. 6B) or *P. dendritiformis T* in this shape onto an agar dish, and allowed the strains to grow at 37°C. After 1 day of growth, we stained the agar dishes for visualization (Fig. 6C, D) as a demonstration of the successful screen printing of different species in the pattern created by the original strain. Using screen printing, a variety of bacteria subtypes or mixtures, thereof, could be patterned on multiple agar conditions to determine the resulting growth patterns.

Discussion

Although it was initially adopted by chain stores for advertisements and sign reproductions, screen printing developed into a fine art form and eventually was adopted by many other commercial industries.[41] Today, screen printing still occupies a unique niche typically recognized for its versatile nature and low capital investment. The method can also print highly viscous and concentrated solutions, which is difficult with other approaches, and allows for varied and consistent thicknesses of layers to be applied additively to surfaces. These features have led to the widespread use of screen printing for manufacturing of solar cells, electrochemical sensors, electroconductive paste, and flexible electronics.[42–44] Although screen printing is a well-adopted technique in many industries, less attention has been devoted to the screen printing of living cells.

The unique requirements of screen printing living cells include biocompatibility and patterning on a surface that allows for

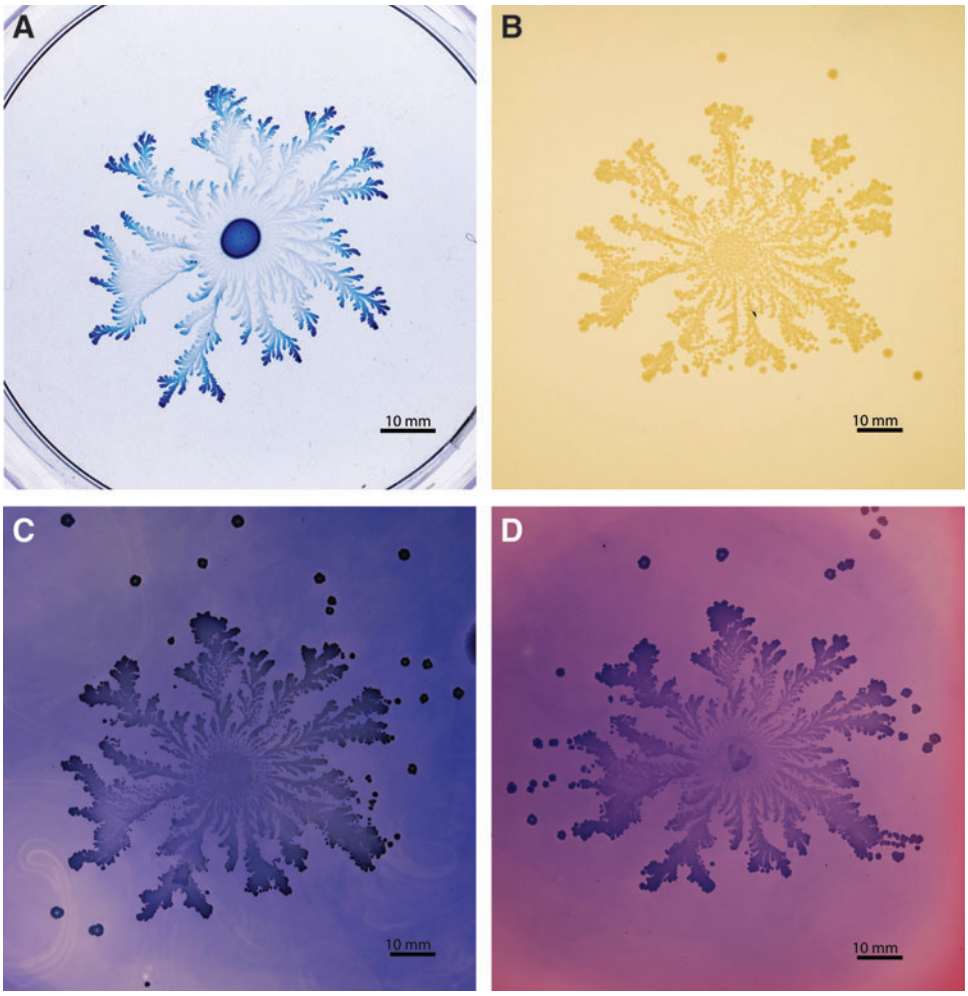

FIG. 6. Screen printing biofilm patterns on agar. **(A)** *Paenibacillus dendritiformis T* bacteria grown on pattern-forming media and stained with Coomassie blue. We used this image as the initial starting point for the biofilm screen design. **(B)** *amilGFP* bacteria screen printed in the biofilm design and grown on agar for 2 days. **(C, D)** *P. dendritiformis T* bacteria patterned, grown at 37°C, and then stained with either **(C)** Coomassie blue or **(D)** Coomassie blue, and methyl red.

cell function or growth. In this work, we have described medium compositions for screen printing bacteria onto agar, paper, and glass surfaces. We demonstrated resolution that is sufficiently high for a variety of studies, using low-cost and quickly produced screens. If less resolution is sufficient, screens can be produced with do-it-yourself (DIY) kits that are readily available and still would produce submillimeter resolution in theory. Since some interactions between living organisms occur at even smaller scales, higher resolution may be obtained by adoption of ultrafine screen printing (6 μm resolution; Kuroda Electric, Czech Republic) or photolithographic approaches for creating the stencils.[45]

Our methodology was able to produce consistent line thicknesses of $\sim$200 μm using a 305 mesh count screen (with $\sim$50 μm openings). In our case, we observed that XG produced the best positive resolution and lowest bleeds of the three mediums. We noted from our images that XG seemed to be comparatively cohesive, binding to itself rather than the surfaces tested, potentially contributing to its low bleed. In contrast, both CMC and GA produced larger bleeds and positive resolutions. However, as we demonstrated, these seem to be at similar levels to commercial screen printing inks, and are expected to some degree in a normal screen printing process. Modifying the chemical properties of these mediums specifically for desired surfaces may allow for greater resolution to be obtained. In addition, further improvements to our experimental setup may improve performance. For instance, commercial screen printing processes use vacuum pressure to keep the screen in close contact with the surface while screen printing. This prevents the screen from shifting laterally or out of the plane, which helps avoid ink from accumulating underneath the screen during the process.

A number of other factors are known to affect the resolution of screen printing. Previous studies have demonstrated that mesh material, medium rheology, squeegee attack angle, squeegee blade characteristic, squeegee speed, and surface variations all play a role in the resulting pattern produced.[46,47] Factors such as the force and speed may be more restrictive in the case of screen printing living cells. For example, agar surfaces tend to be soft and require lower pressures when screen printing, and some cell types are sensitive to shear stress.[48] Alternatively, screen printing on temporary substrates that can be transferred to agar dishes may offer an increased flexibility. Subsequent studies providing a rigorous analysis of screen printing variables will optimize the process for living cells, and could be improved by mechanical and robotic control of the screen printing process.

Screen printing also has several benefits in terms of the scalability of printing live cells. As screen printing is used in a variety of industries such as electronics and textile industries, the infrastructure in place could readily be used to print bacteria instead of conventional inks. Screen sizes can reach up to 100–120″, can print a large array of colors (or types of bacteria), and can reach up to 1500–4500 sheets per hour for linear and cylindrical screen printing, respectively. One major advantage is that prints can be made on almost any material including paper, textiles, wood, ceramics, glass, and leather. Furthermore, screen printing can be adapted to irregular and nonflat surfaces, while maintaining consistent printing.[49,50] At a small scale, these properties allow for rapid iteration and prototyping of various surfaces, suspension mediums, bacterial strains, and patterns. In terms of product management on a large scale, maintenance, repairs, and new components for screen printing are often more economical and simple than digital technology that has frequent software and hardware updates and rapid platform obsolescence. In comparison, microfabrication approaches are typically higher resolution, but have smaller print sizes, and may require special facilities and material handling. Current bioprinting approaches are capable of relatively small size prints and are typically used for research purposes. Large-scale inkjet printers could be modified to print bacteria; however, additional complications in clogging of print heads, thermal technology that may cause loss of bacterial viability, and a smaller set of surfaces to print on are drawbacks that may be limiting in comparison to screen printing.

Lastly, use of the screen printing system also readily lends itself to the field of ''Bioart.'' Bioart is a growing field within the art world, with examples including use of recombinant DNA, painting with bacteria, and microscopic imagery that is immunohistochemically stained or colored.[51] While bacteria have been used for bioart in many different projects, the art is usually created through traditional scientific methods that may be difficult to gain access to or are challenging to manipulate with precision. In this study, we developed a screen printing system that is familiar and particularly suited to artists, and can be adapted to print a variety of living organisms on different surfaces. This simple approach can be utilized by both artists and scientists alike and used as a platform for engagement in education and science literacy.

Conclusion

In this article, we described a process to screen print living bacteria in precise spatial configurations onto agar, paper, and glass surfaces. We demonstrated how different applications ranging from printing bacteria biosensors on paper to biofilm pattern formation could benefit from our single or multipopulation screen printing approach. The advantage of the technique described in this study lies primarily in its versatile nature, allowing users to prototype designs and adopt existing infrastructure, tools, and knowledge from industrial screen printing technology. Furthermore, screen printing is a highly scalable technology and can be extended to manufacturing on a large scale. In the future, we hope to rigorously develop this system to allow for higher resolutions, controlled thicknesses, and antibiotics to prevent bacterial growth.

Acknowledgment

This work was supported, in part, by the NIH Pathway to Independence Award (R00CA197649-02).

Author Disclosure Statement

No competing financial interests exist.

References

1. D'Souza SF. Microbial biosensors. Biosens Bioelectron 2001;16:337–353.
2. Bousse L. Whole cell biosensors. Sensor Actuat B-Chem 1996;34:270–275.
3. Danino T, *et al.* Programmable probiotics for detection of cancer in urine. Sci Transl Med 2015;7:289–284.
4. Yu YA, *et al.* Visualization of tumors and metastases in live animals with bacteria and vaccinia virus encoding light-emitting proteins. Nat Biotechnol 2004;22:313–320.
5. Brader P, *et al. Escherichia coli* Nissle 1917 facilitates tumor detection by positron emission tomography and optical imaging. Clin Cancer Res 2008;14:2295–2302.

6. Gupta S, Bram EE, Weiss R. Genetically programmable pathogen sense and destroy. ACS Synth Biol 2013;2:715–723.
7. Hwang IY, *et al.* Reprogramming microbes to be pathogen-seeking killers. ACS Synth Biol 2013;3:228–237.
8. Moon TS, Lou C, Tamsir A, Stanton BC, Voigt CA. Genetic programs constructed from layered logic gates in single cells. Nature 2012;491:249–253.
9. Danino T, Mondragon-Palomino O, Tsimring L, Hasty J. A synchronized quorum of genetic clocks. Nature 2010;463: 326–330.
10. Basu S, Gerchman Y, Collins CH, Arnold FH, Weiss R. A synthetic multicellular system for programmed pattern formation. Nature 2005;434:1130–1134.
11. Tabor JJ, *et al.* A synthetic genetic edge detection program. Cell 2009;137:1272–1281.
12. Tamsir A, Tabor JJ, Voigt CA. Robust multicellular computing using genetically encoded NOR gates and chemical 'wires'. Nature 2011;469:212–215.
13. Liana DD, Raguse B, Gooding JJ, Chow E. Recent advances in paper-based sensors. Sensors 2012;12:11505–11526.
14. Martinez AW, Phillips ST, Whitesides GM, Carrilho E. Diagnostics for the developing world: Microfluidic paper-based analytical devices. Anal Chem 2009;82:3–10.
15. Pardee K, *et al.* Paper-based synthetic gene networks. Cell 2014;159:940–954.
16. Bhatia SN, Yarmush ML, Toner M. Controlling cell interactions by micropatterning in co-cultures: Hepatocytes and 3T3 fibroblasts. J Biomed Mater Res 1997;34:189–199.
17. Weibel DB, DiLuzio WR, Whitesides GM. Microfabrication meets microbiology. Nat Rev Microbiol 2007;5:209–218.
18. Stevens KR, *et al.* InVERT molding for scalable control of tissue microarchitecture. Nat Commun 2013;4:1847.
19. Mironov V, Reis N, Derby B. Review: Bioprinting: B beginning. Tissue Eng 2006;12:631–634.
20. Todhunter ME, *et al.* Programmed synthesis of three-dimensional tissues. Nat Methods 2015;12:975–981.
21. Yao L, *et al.* bioPrint: A liquid deposition printing system for natural actuators. 3D Print Addit Manuf 2015; 2:168–179.
22. Cohen DJ, Morfino RC, Maharbiz MM. A modified consumer inkjet for spatiotemporal control of gene expression. PLoS One 2009;4:e7086.
23. Nakamura M, *et al.* Biocompatible inkjet printing technique for designed seeding of individual living cells. Tissue Eng 2005;11:1658–1666.
24. Xu T, *et al.* Viability and electrophysiology of neural cell structures generated by the inkjet printing method. Biomaterials 2006;27:3580–3588.
25. Xu T, Jin J, Gregory C, Hickman JJ, Boland T. Inkjet printing of viable mammalian cells. Biomaterials 2005;26:93–99.
26. Krebs FC. Fabrication and processing of polymer solar cells: A review of printing and coating techniques. Sol Energ Mat Sol C 2009;93:394–412.
27. Tudorache M, Bala C. Biosensors based on screen-printing technology, and their applications in environmental and food analysis. Anal Bioanal Chem 2007;388:565–578.
28. Li M, Li Y-T, Li D-W, Long Y-T. Recent developments and applications of screen-printed electrodes in environmental assays—a review. Anal Chim Acta 2012;734:31–44.
29. Gilleo K. Rheology and surface chemistry for screen printing. Screen Print Magaz 1989;128–132.
30. Zatz JL, Knapp S. Viscosity of xanthan gum solutions at low shear rates. J Pharm Sci 1984;73:468–471.
31. Li X, *et al.* Rheological properties of gum arabic solution: From Newtonianism to thixotropy. Food Hydrocoll 2011; 25:293–298.
32. Benchabane A, Bekkour K. Rheological properties of carboxymethyl cellulose (CMC) solutions. Colloid Polym Sci 2008;286:1173–1180.
33. Jacob EB, Becker I, Shapira Y, Levine H. Bacterial linguistic communication and social intelligence. Trends Microbiol 2004;12:366–372.
34. Budding AE, Ingham CJ, Bitter W, Vandenbroucke-Grauls CM, Schneeberger PM. The Dienes phenomenon: Competition and territoriality in swarming Proteus mirabilis. J Bacteriol 2009;191:3892–3900.
35. Be'Er A, *et al.* Deadly competition between sibling bacterial colonies. Proc Natl Acad Sci 2009;106:428–433.
36. Budrene EO. Berg HC. Complex patterns formed by motile cells of *Escherichia coli*. Nature 1991;349:630–633.
37. Woodward DE, *et al.* Spatio-temporal patterns generated by *Salmonella typhimurium*. Biophys J 1995;68;2181.
38. Ben-Jacob E, *et al.* Generic modelling of cooperative growth patterns in bacterial colonies. Nature 1994;368:46–49.
39. Fujikawa H, Matsushita M. Fractal growth of Bacillus subtilis on agar plates. J Phys Soc Jpn 1989;58:3875–3878.
40. Ben-Jacob E, Shmueli H, Shochet O, Tenenbaum A. Adaptive self-organization during growth of bacterial colonies. Phys A 1992;187:378–424.
41. Lengwiler G. A History of Screen Printing: How an Art Evolved Into an Industry. Cincinnati, OH: ST Media Group International, 2013; p.484.
42. Ostfeld AE, Deckman I, Gaikwad AM, Lochner CM, Arias AC. Screen printed passive components for flexible power electronics. Sci Rep 2015;5:1–11.
43. Brenner P. Printing photovoltaics. Industr Special Print 2010;1:26–33.
44. Albareda-Sirvent M, Merkoci A, Alegret S. Configurations used in the design of screen-printed enzymatic biosensors. A review. Sensor Actuat B-Chem 2000;69:153–163.
45. Hyun WJ, Secor EB, Hersam MC, Frisbie CD, Francis LF. High-resolution patterning of graphene by screen printing with a silicon stencil for highly flexible printed electronics. Adv Mater 2015;27:109–115.
46. Austin BM. Thick-film screen printing. Solid State Technol 1969;12:53–58.
47. Bacher RJ. High-resolution thick-film printing. Screen Print 1994;84:104–107.
48. Ingber DE. Cellular mechanotransduction: Putting all the pieces together again. FASEB J 2006;20:811–827.
49. Kokot J. UV Technology: A Practical Guide for All Printing Processes. Wiesbaden, Germany: Berufsgenossenschaft Druck und Papierverarbeitung, 2007.
50. Kipphan H. Handbook of Print Media: Technologies and Production Methods. Berlin: Springer Science & Business Media, 2001.
51. Yetisen AK, Davis J, Coskun AF, Church GM, Yun SH. Bioart. Trends Biotechnol 2015;33:724–734.

Address correspondence to:
Tal Danino
Department of Biomedical Engineering
Columbia University
550 West 120th St.
Northwest Corner Building
New York, NY 10027

E-mail: td2506@columbia.edu