FULL PAPER

Synthetic Biology

ADVANCED FUNCTIONAL MATERIALS

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Light-Controlled, High-Resolution Patterning of Living Engineered Bacteria Onto Textiles, Ceramics, and Plastic

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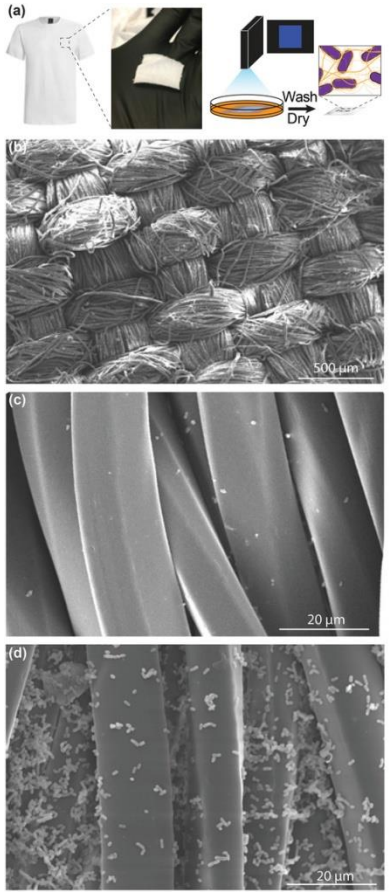

Introduction

- Light is used to pattern *Escherichia coli* onto diverse materials by controlling the expression of curli fibers that anchor the formation of a biofilm.
- The adhered cells are demonstrated to respond to sensory information, including small molecules (IPTG and DAPG) and light from light-emitting diodes.
- By projecting color images onto the material containing bacteria, this system can be used to pattern the growth of composite materials, including layers of protein and gold nanoparticles.
- Changes in gene expression have been reported to be induced as quickly as 10 s and at 3 μm resolution.

Significance

SCIENCE ADVANCES | RESEARCH ARTICLE

MATERIALS SCIENCE

3D printing of bacteria into functional complex materials

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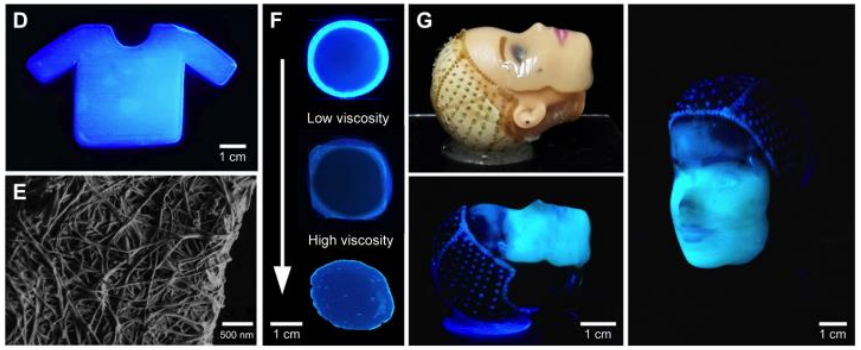
December 2017

Manuel Schaffner,[1]* Patrick A. Rühs,[1]*† Fergal Coulter,[1,2] Samuel Kilcher,[3] André R. Studart[1]†



RESEARCH ARTICLE | BIOENGINEERING

Harnessing the hygroscopic and biofluorescent behaviors of genetically tractable microbial cells to design biohybrid wearables

Wen Wang[1,2], Lining Yao[2], Chin-Yi Cheng[2,3], Teng Zhang[4], Hiroshi Atsumi[5], Luda Wang[4], Guanyun Wang[2], Oksana Anilionyte…

+ See all authors and affiliations

Science Advances 19 May 2017:
Vol. 3, no. 5, e1601984
DOI: 10.1126/sciadv.1601984

Relevance

- 3D printing of Bacteria
- Wearable Sensors

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Harnessing the hygroscopic and biofluorescent behaviors of genetically tractable microbial cells to design biohybrid wearables

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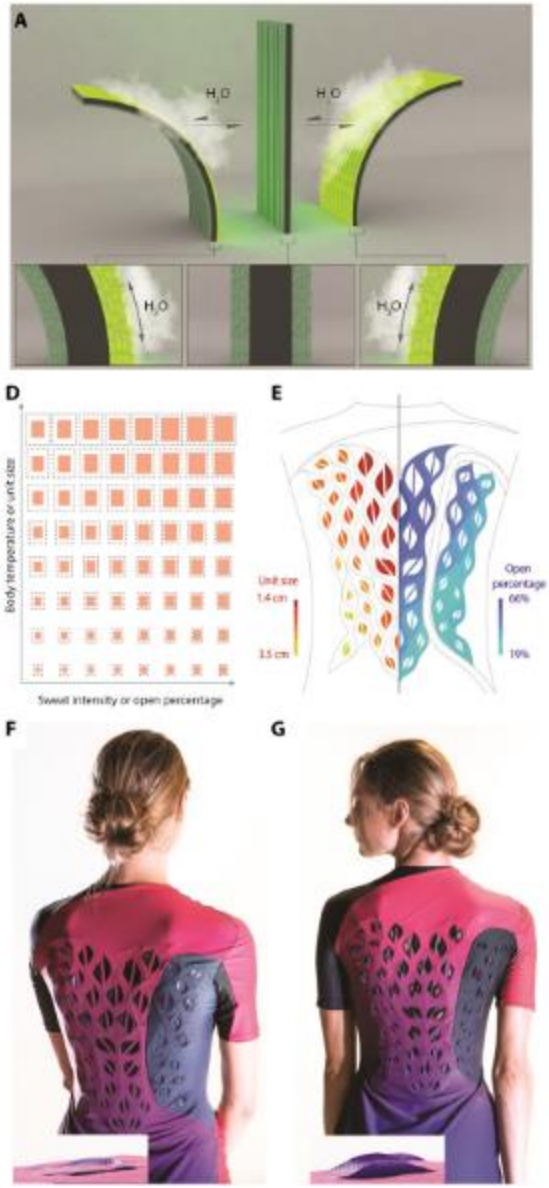


•A 22-gene genetic system that simultaneously controls the production of three variants of CsgA in response to red, green, and blue light was designed.

• In the RGB system, colored light is perceived by three light sensors based on phytochromes (red/green) and an light-oxygen-voltage (LOV) domain protein (blue).

• Each color signal then activates production of one of three different T7 RNA polymerase (RNAP) variants that in turn activate their cognate promoter.

•In the system reported here, each T7 promoter controls either wild-type (WT) CsgA or a codon shuffled variant fused to a different affinity tag (HA or His)

•The genes involved in curli synthesis are encoded in two operons: *csgBAC* and *csgDEFG*. The *csgDEFG* operon is tightly regulated by stress responsive transcription factors and is induced in nitrogen-poor media at low temperature (30 °C)

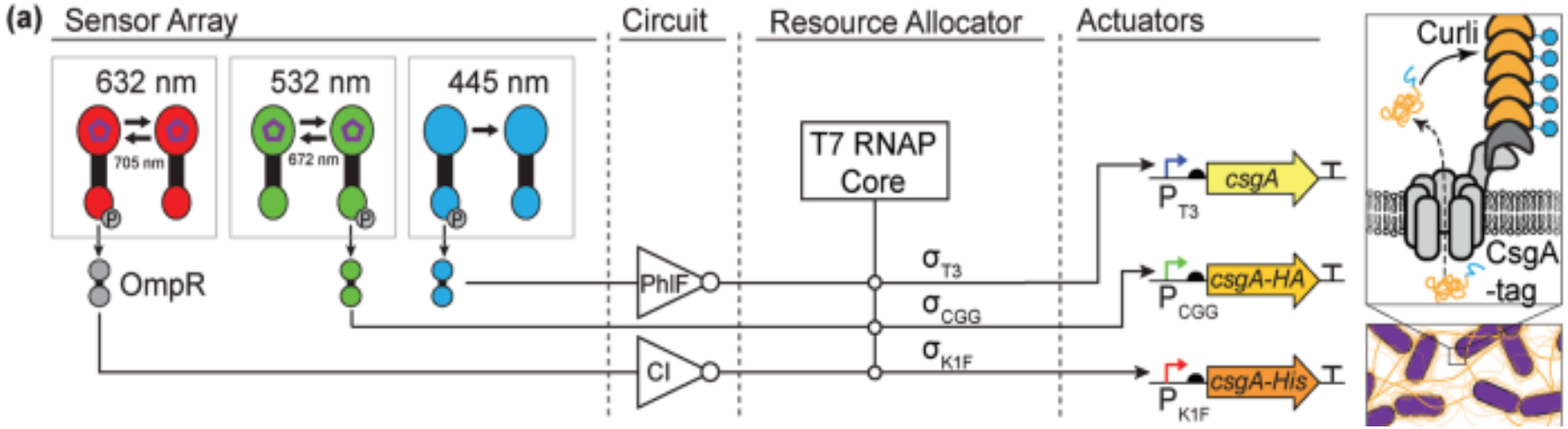
•CsgD is a transcription factor that stimulates transcription of *csgBAC*.

•CsgA is secreted into the extracellular medium in a soluble form and the surface-localized CsgB nucleates its polymerization into curli fbers.

•CsgC is thought to prevent premature curli formation in the cytoplasm.

•CsgE, CsgF, and CsgG together comprise the secretion apparatus that selectively transports CsgB and CsgA to the outer membrane of the cell. CsgA has been engineered to display peptide tags, which in turn have been used to recruit gold nanoparticles and enzymes to functionalize the fibers.

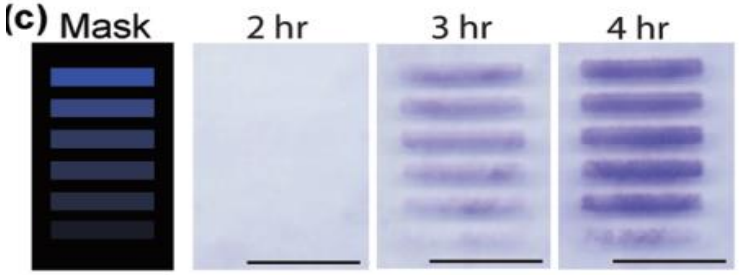
- To prevent cross-talk between the engineered system and the native curli system, a knockout of the native operons was made in *E. coli* JF1 (Δ*csg).*
- The *csgBAC* operon was then placed under control of PT3 (blue light) and recoded variants of CsgA fused to the HA or His affinity tags were placed under the control of PCGG (green light) and PK1F (red light), respectively.
- All *csg* genes were encoded on a pSC101 plasmid (pFM1300), which was co-transformed into *E. coli* JF1 Δ*csg* with the plasmids encoding the RGB system (pJFR1, pJFR2, and pJFR3).



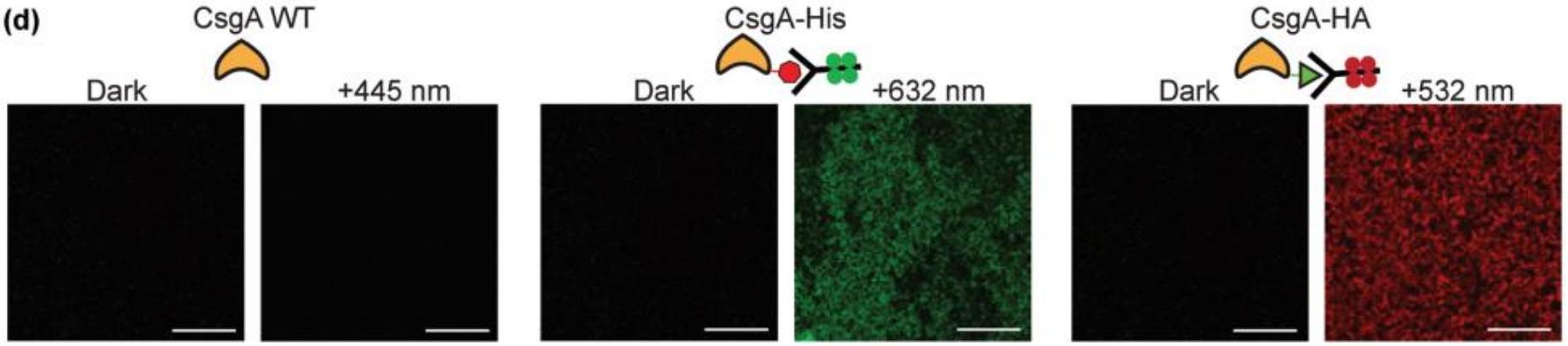
Schematic of the optogenetic control system that drives curli biofilm formation.

- The ability to induce adhesion of cells to the surface of a material in response to colored light.
- Diluted an overnight culture of *E. coli* JF1Δ*csg* containing the light inducible system into liquid Luria–Bertani (LB) media and added the diluted culture into a sterile polystyrene petri plate. This plate was placed in a 37 °C incubator and a pattern of colored light was projected onto it by a commercial light-emitting diode (LED) projector.
- Following incubation for 18 h, unattached cells were washed away with sterile water, revealing a white film in the shape of the projected pattern. This pattern was strongly stained by crystal violet, indicating that the film was largely composed of cells.

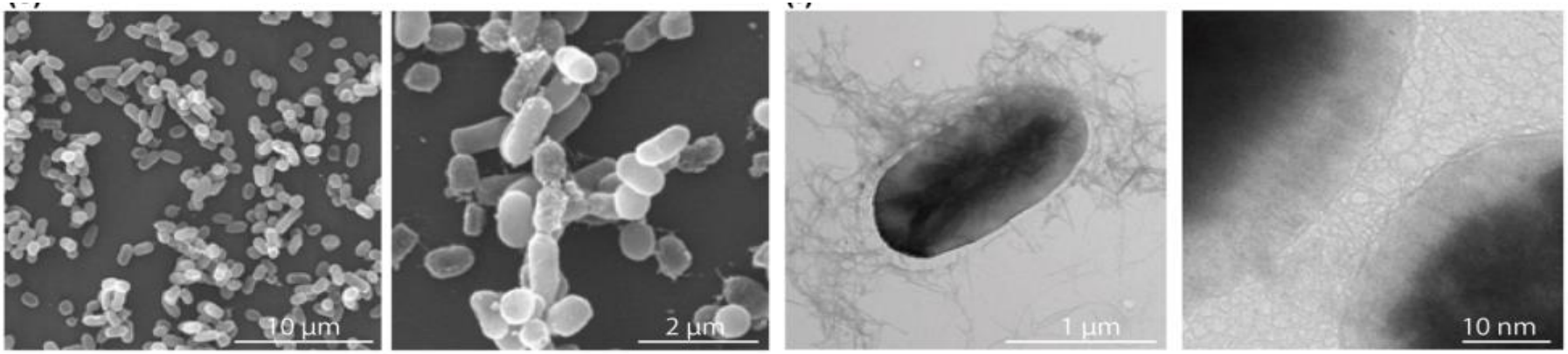
Schematic of the experimental setup for generating light-induced bioflms. The shown three-color pattern was projected onto a plate containing the RGB curli strain suspended in media. The resulting bioflm was washed and stained with crystal violet. In this strain, blue light induces untagged csgA, red light induces His-tagged csgA, and green light induces HA-tagged csgA. The scale bar is 1 cm.



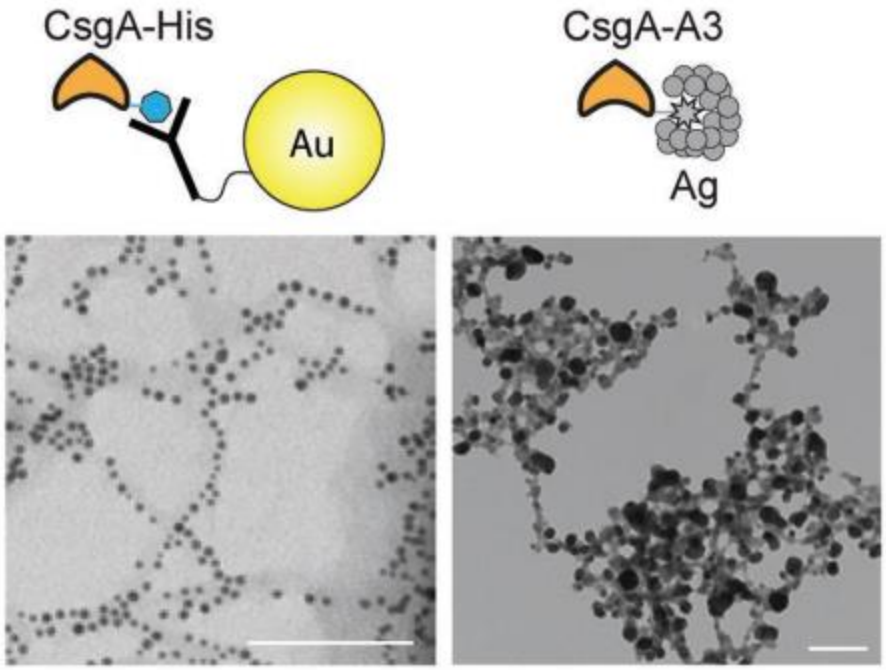
Timed generation of curli bioflm. The scale bars are 1 cm



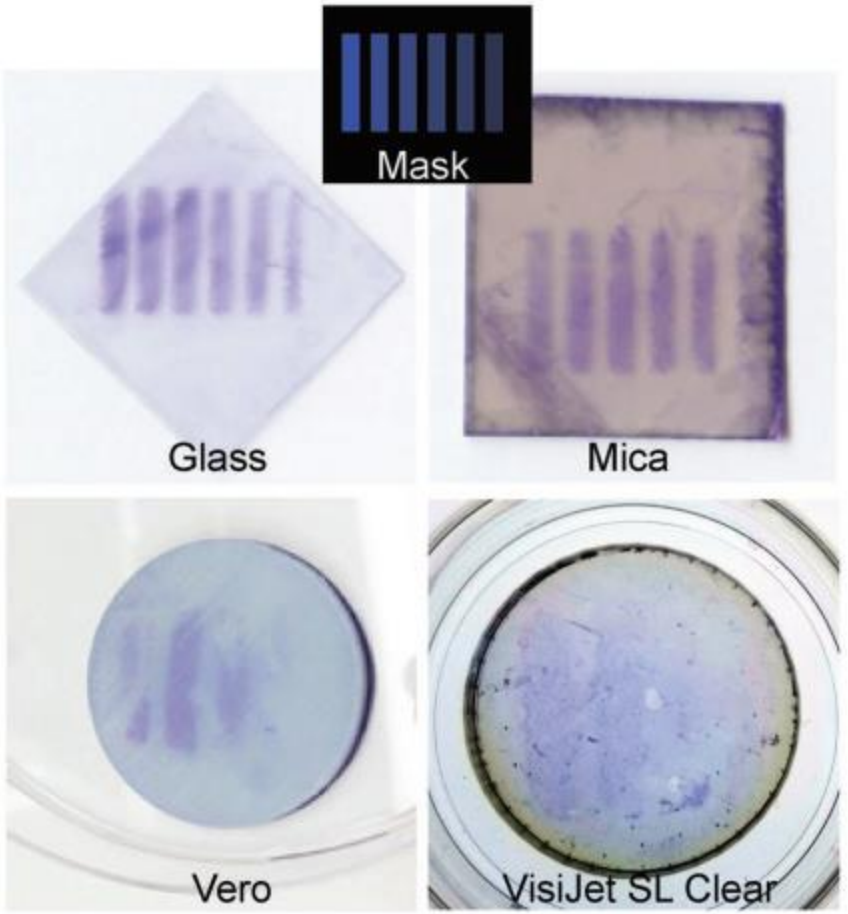
Each bioflm generated by the three-color system was simultaneously stained with FITC-conjugated antibody and PE-TxRed-conjugated antibody and then imaged by fluorescence microscopy. The untagged (blue light, 470 nm) csgA-WT biofilm is shown on the left and is not expected to bind antibodies, the csgA-12xHis (red light, 632 nm) biofilm is shown in the middle, and the csgA-HA (green light, 532 nm) biofilm is shown on the right. Dark regions of plate were imaged for comparison. Scale bars are 30 µm.



SEM images of the biofilm after 3 hours of growth. TEM images of cells in the biofilm show a fine mesh of nanofibers that aggregate into larger fibers. Data are representative of three independent experiments done on different days.

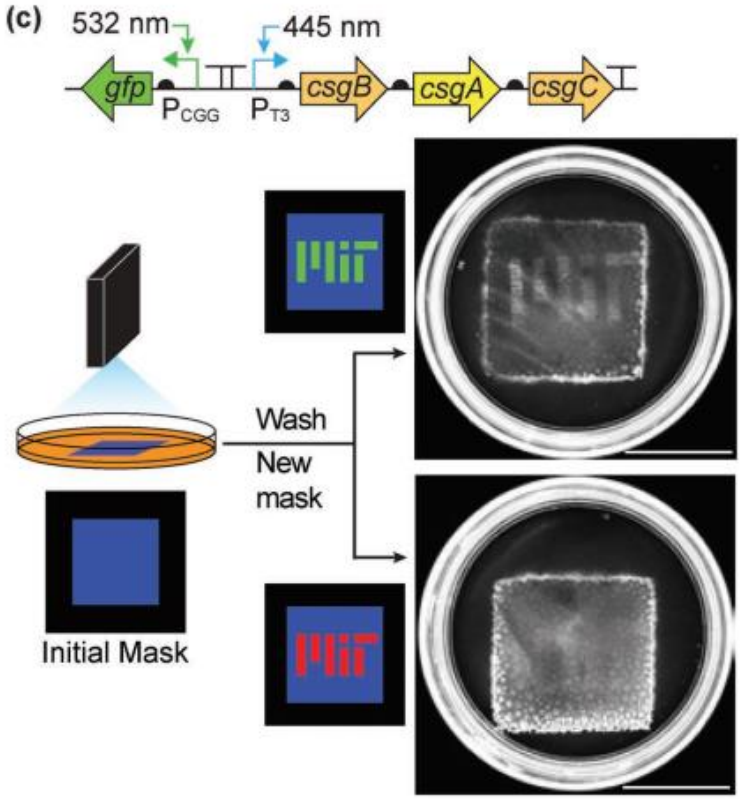


CsgA-12xHis curli fibers (left) were labeled with 5 nm diameter NiNTA-AuNPs and imaged with TEM. NiNTA-AuNPs can be seen as black dots along the fibers. CsgA-A3 peptide curli bioflms (right) were washed and then incubated with aqueous AgNO3 (150 × 10-3 m) for 6 h. The biofilm was then washed with water, scrapped from the plate, and placed on a TEM grid (Methods). The resulting CsgA-A3 curli fibers contained variably sized nanoparticles. Scale bars are 100nm.



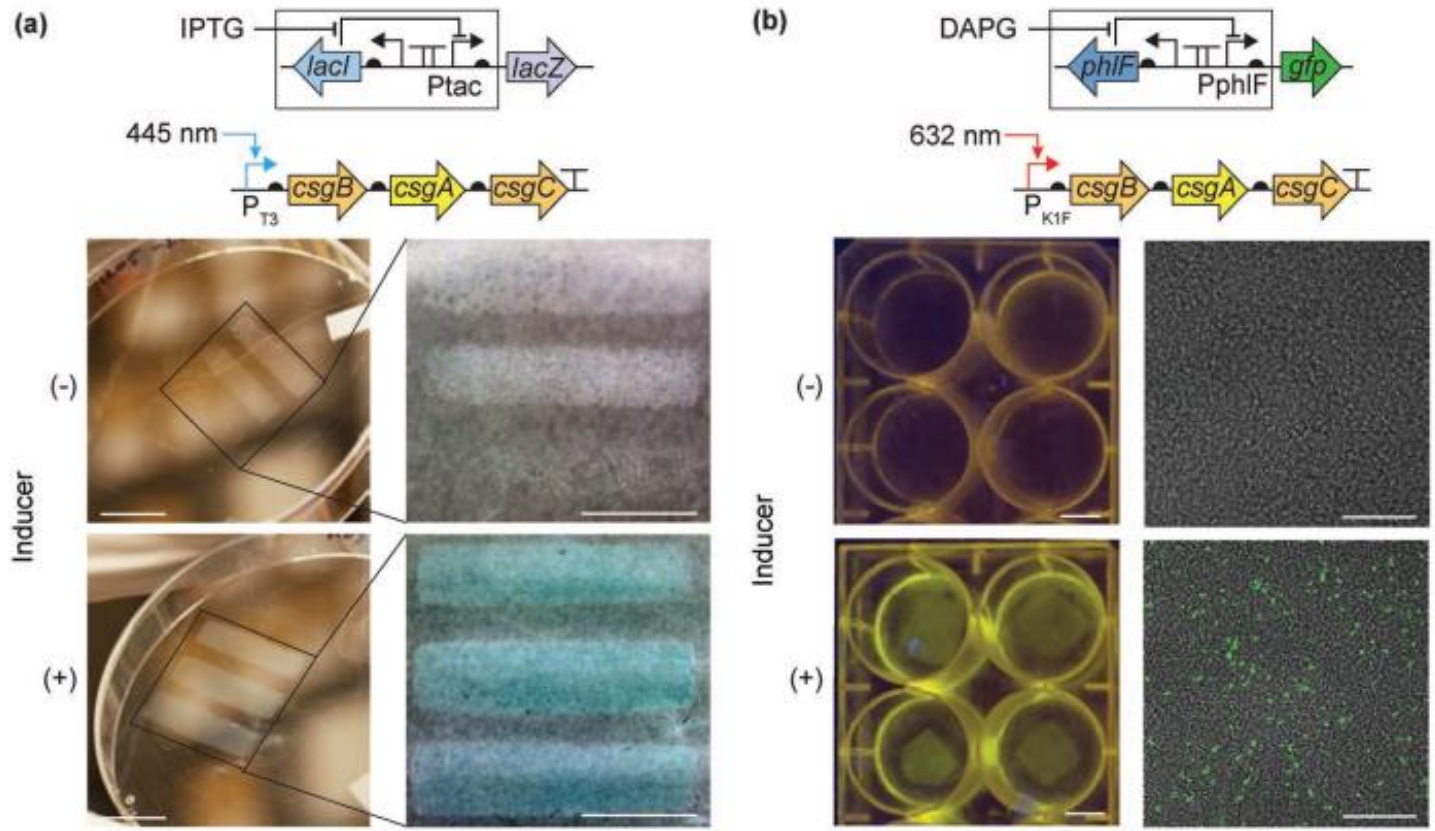
Blue light-induced WT curli strains can form biofilms on various materials, including glass, mica, and 3D-printed plastics (Vero and VisiJet SL Clear). The VisiJet SL Clear image was modified in Adobe Photoshop to improve visualization.

Biofilm-Embedded Cells Respond to Environmental Signals



Genetic diagram for a plasmid (pFM1320) encoding a curli output that generates GFP in response to green light. The biofilm is initially formed by projecting a blue square onto the plate. Following washing and addition of fresh media, either a green or red pattern (inset) is projected onto the biofilm for 4 h. The biofilm is then imaged for GFP production (methods). The red pattern serves as a negative control. Scale bars are 1 cm.

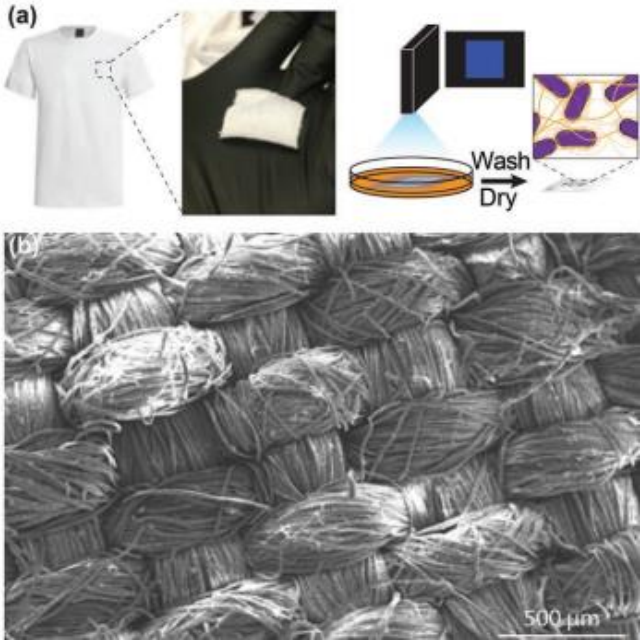
Light-patterned biofilms are robust biosensors.



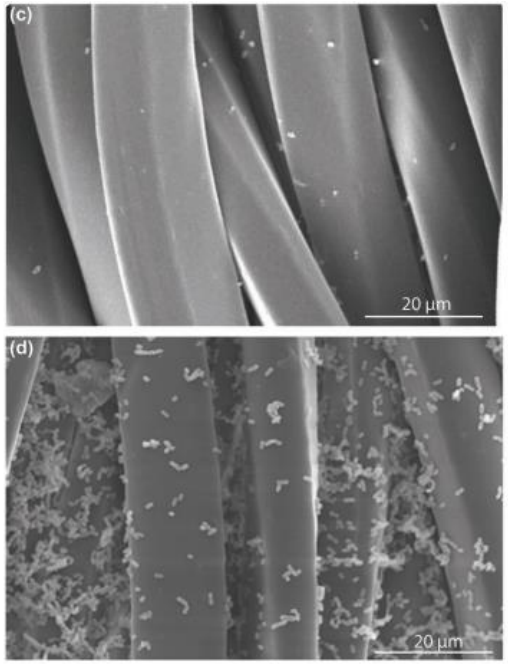
Shown is the genetic diagram of the Blue Only curli strain that generates β-galactosidase in response to IPTG. Bioflims formed by this strain were exposed to X-gal and to either no IPTG (top) or 1×10^{-3} M IPTG (bottom). Scale bars are 1 cm

Genetic diagram of the Red Only curli strain that generates GFP in response to DAPG(2,4-diacetylphloroglucinol). Biofilms formed by this strain were exposed to either no DAPG (top) or 25×10^{-6} M DAPG (bottom). GFP in the biofilm was imaged on a blue light transilluminator (left) and by fluorescent microscopy (right). Error bars are 1 cm in the left images and 50 µm in the right images.

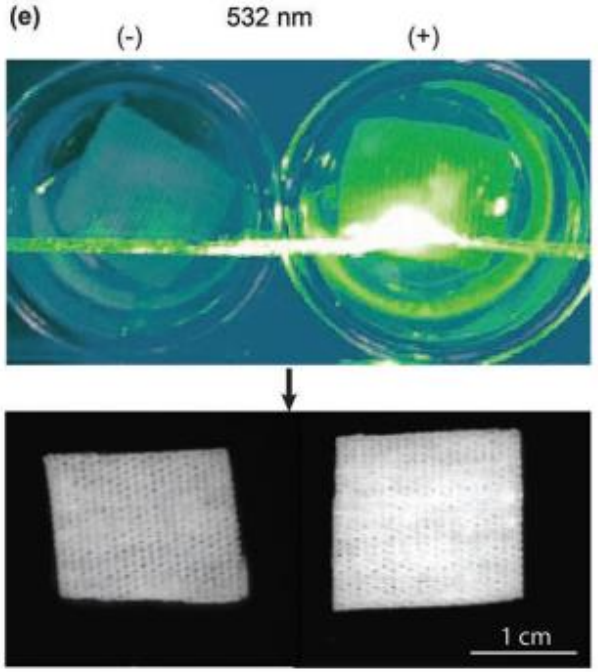
Biofilms Integrate Cellular Functions on Wearable Fibers



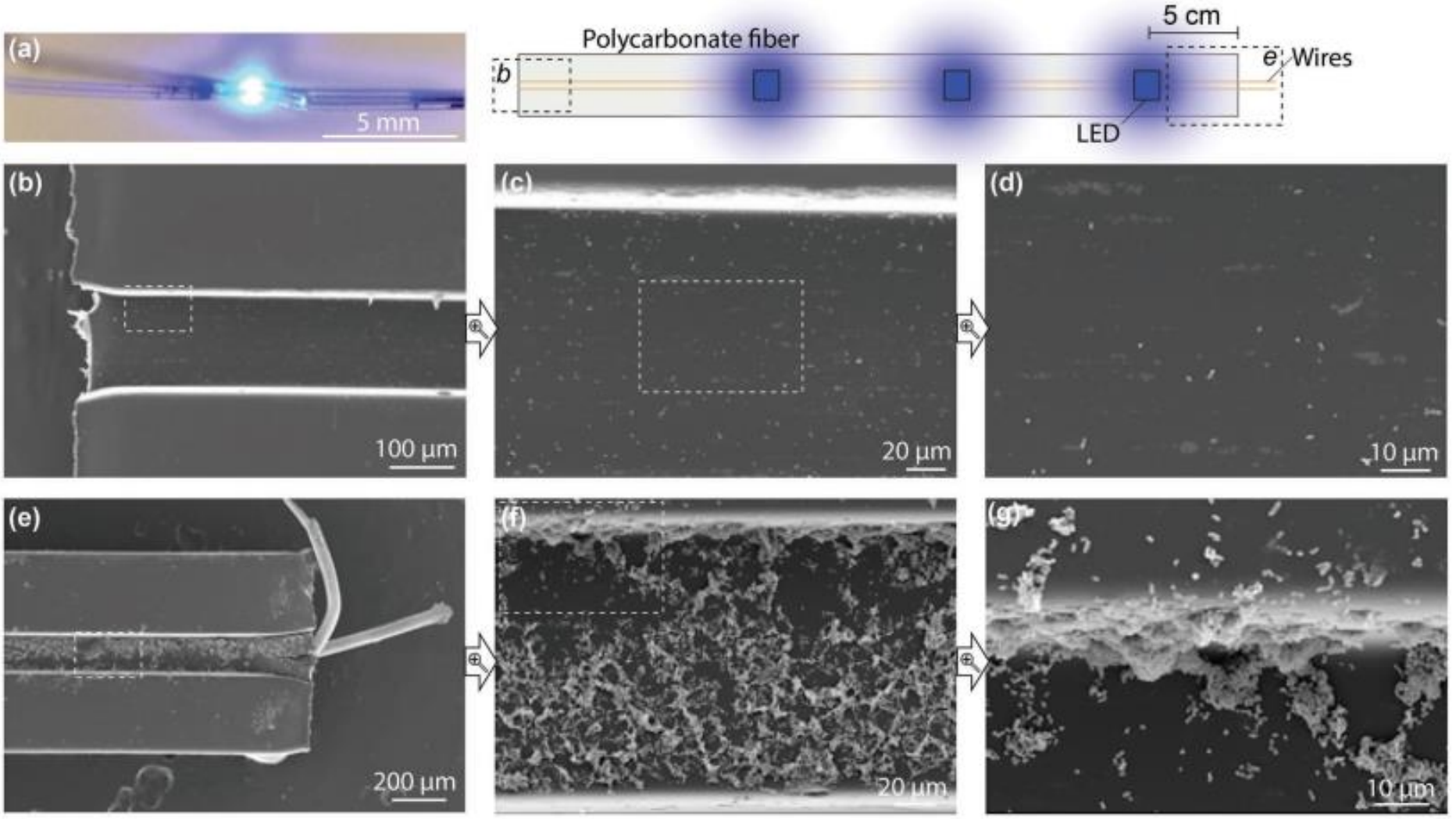
A cotton t-shirt was sectioned into 2 × 2 cm fabric squares. These squares were immersed in a culture expressing WT CsgA in response to blue light and GFP in response to green light. Following exposure to blue light from a projector for 6 h, the squares were washed, dried, and prepared for SEM.



SEM of a fabric square that was exposed to blue light.



Following blue light exposure and washing, some fabric squares were either placed directly under an LED fiber producing green light (532 nm) or at least 2 cm away. The squares were then imaged for GFP production under blue transillumination.



a) A picture and diagram of a polycarbonate fiber containing regularly spaced LEDs (20 cm apart) and tungsten wires for conduction. An active fiber was incubated with a culture of a blue-only curli strain, washed, and then sectioned for analysis. b–d) SEM micrographs with increasing magnification (left to right) of a site 5 cm away from the nearest LED. e–g) SEM micrographs with increasing magnification (left to right) of a site and a site near an LED.

Conclusion

- Engineered cells to form living coatings whose adherence to the outer surface of a variety of materials can be controlled with light.
- The adhered bacteria remain alive and responsive to chemical and light signals following extensive mechanical washing.
- The incorporation of such sense-and respond functionalities into materials, particularly wearable devices and clothing, shows great promise.

Thank you