DIAGNOSTICS

Textile-embedded cell-free biosensors

Freeze-dried genetic circuits can be integrated with textiles for the detection — colorimetric, or via fluorescence or luminescence — of small molecules and nucleic acids from SARS-CoV-2 and other pathogens.

Yuan Lu

Living cells with programmed genetic circuits can make excellent biosensors. Yet, functioning cells naturally require adequate nutrient supply and suitable environmental conditions (with regards to oxygen concentration, temperature and humidity, in particular), and genetically modified organisms can raise safety considerations that constrain their use[1]. In addition, because the target analytes have to cross cellular membranes, cell-based biosensors can have slow response times. The analytes themselves can also be toxic to the cells. However, DNA templates and the transcriptional and translational machinery can be adapted for use outside of the cell environment, thus facilitating the manipulation of the reactions, lessening biosafety concerns, expanding the range of compatible substrates, and making the genetic circuits more tolerant to toxicity[2].

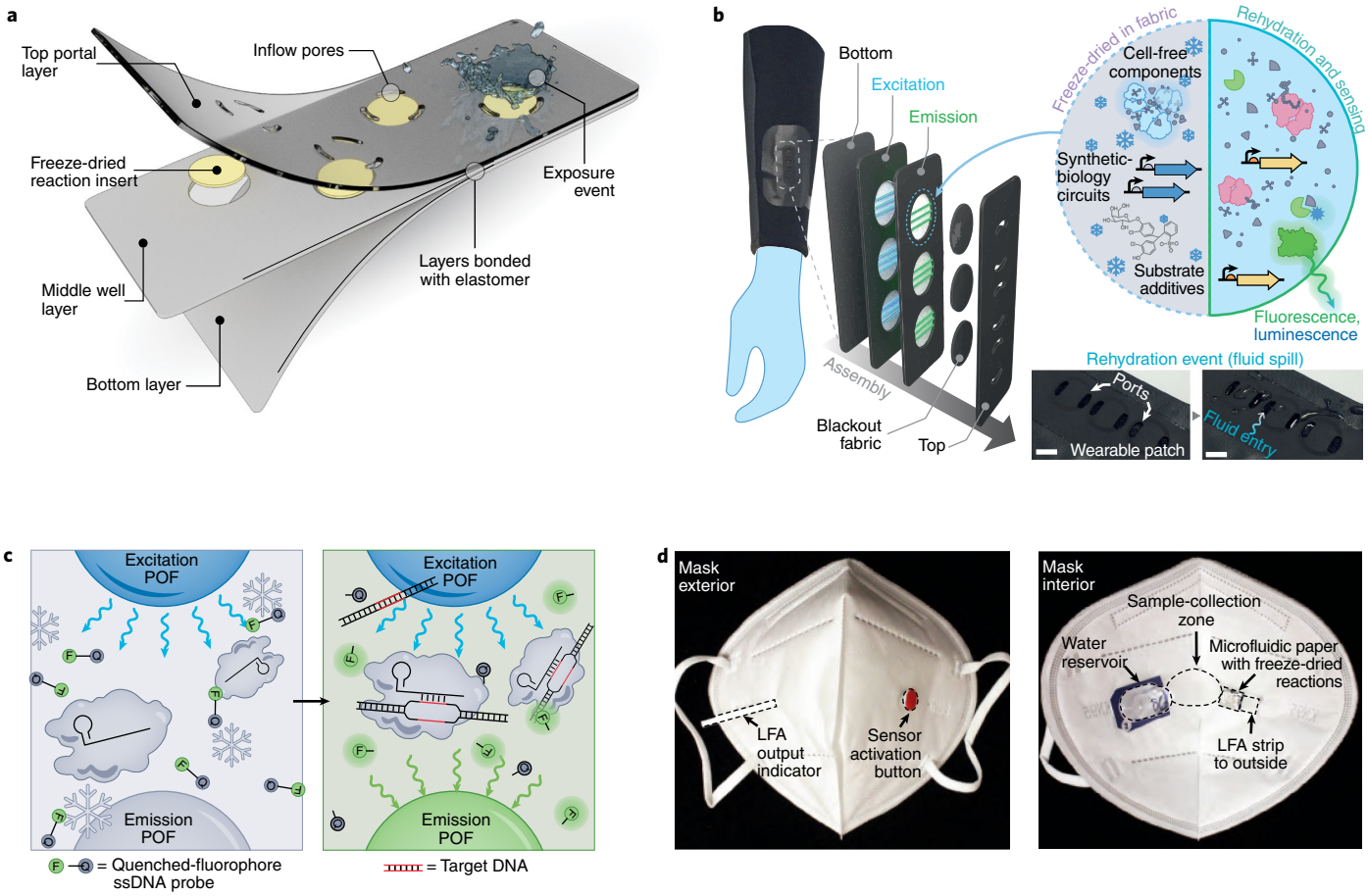

Fig. 1 | Wearable textile-embedded biosensors relying on freeze-dried cell-free synthetic biology. a, Assembly of a three-layer elastomeric device, with a middle layer containing freeze-dried reaction insets embedded in a matrix of cellulose. **b**, A wearable textile with integrated freeze-dried cell-free biosensors (upper right) and interweaved with polymeric optic fibres for excitation and emission. Rehydration leads to the wicking of the sample through ports into the reaction wells (bottom right). Scale bars, 5 mm. **c**, Nucleic-acid sensing via Cas12a-guide-RNA and the recombinase polymerase amplification of a target double-stranded DNA sequence by Cas12a, followed by the collateral cleavage of single-stranded DNA (ssDNA) probes bound to a fluorophore. POF, polymeric optic fibre. **d**, A face mask embedding a biosensor for the detection of SARS-CoV-2 RNA. The mask is fitted with a water reservoir for rehydration, a sample-collection zone, the paper-based biosensor with freeze-dried cell-free reagents, and a lateral flow assay (LFA) strip for nucleic-acid detection. Figure reproduced with permission from ref. [4], Springer Nature America, Inc.

Such cell-free synthetic-biology systems can be freeze-dried for long-term storage[3]. James Collins and colleagues recently reported in *Nature Biotechnology* that freeze-dried cell-free synthetic biological circuits can be integrated with textiles[4], and that the textile-embedded circuits can be designed to detect small molecules and nucleic acids on rehydration.

Collins and co-authors used silicone elastomers and cellulose matrices to assemble a flexible three-layer device, incorporating freeze-dried insets that contain the genetic circuits and are connected fluidically to inflow ports (Fig. 1a). The genetic circuits employed the commonly used *lacZ* β-galactosidase operon — that is, a cluster of genes under the control of a single promoter — as the circuit output to hydrolyse a substrate (chlorophenol red-β-D-galactopyranoside) on exposure to a target analyte, causing the substrate to change colour (from yellow to purple). As environmentally exposed reactions can be subject to evaporation after rehydration, inappropriate dilution of the components and variable environmental humidity and temperature, the authors optimized the materials in the device and the kinetics of the cell-free reactions to speed up the colorimetric output to complete in less than 60 minutes after exposure to the analyte. They built prototype devices with freeze-dried circuits for the constitutive expression of *lacZ*, and for the detection of anhydrotetracycline via transcriptional regulation, the RNA of the Ebola virus via a toehold switch, and small molecules via a riboswitch.

To apply the cell-free biosensors to woven fabrics and individual threads, Collins and co-authors conducted a compatibility screen, using more than 100 fabrics, for the immobilization of the freeze-dried cell-free reaction reagents. Moreover, they customized a wearable interweaved polymeric optic fibre, and built a custom wearable spectrometer to monitor signal outputs from the rehydrated lyophilized biosensors via a smartphone app. They showcase the wide applicability of the textile-embedded cell-free biosensors (Fig. 1b) with proof-of-principle applications: the sensing of theophylline via an inducible riboswitch, the activation of a fluorophore via a Broccoli aptamer, the detection of HIV RNA and *Borrelia burgdorferi* RNA via toehold switches, and the detection of organophosphate nerve agents via coupled-enzyme reactions. The authors also integrated the clustered regularly interspaced short palindromic repeats (CRISPR)-based specific high-sensitivity enzymatic reporter unlocking assay[5] (known as SHERLOCK) into the textiles (Fig. 1c), and used them to examine three common resistance markers in *Staphylococcus aureus*. This wearable detection system achieved sensitivities rivalling those of the standard quantitative polymerase chain reaction (qPCR). Furthermore, the authors embedded optical fibres carrying the output emissions of different biosensors into a jacket, and linked a bundled fibre from the fabric to a wearable spectrometer wirelessly connected to a custom smartphone app, for the monitoring of biological hazards in real time.

Collins and collaborators had previously leveraged the freeze-dried cell-free technology to develop paper-based diagnostics for the Ebola[6] and Zika[7] viruses. Collins and co-authors have now integrated such technology into face masks for the detection of SARS-CoV-2 RNA. The face mask houses four modules (Fig. 1d): a water reservoir for rehydration; a sample-collection zone (the fluid accumulates as a result of normal respiration); a paper-based microfluidic analytical device with freeze-dried components for lysis, amplification and sensing; and a lateral flow strip for detection. A readout from the paper-based biosensor in the face mask can be obtained in 1.5 hours, and the detection limit matched that of standard qPCR assays. Notably, the face mask is shelf-stable, functions in ambient conditions and operates autonomously on the press of a button (which pierces a reservoir containing nuclease-free water; the water then flows through the sample-collection zone, the freeze-dried module and the lateral flow strip).

As Collins and co-authors show for soft garments and face masks, the freeze-dried genetic circuits are clearly amenable to integration with different textiles, can employ a range of signal outputs (colorimetric, fluorescence or luminescence), do not require particular skills from the user, and provide rapid results with sensitivity comparable to the sensitivities of traditional laboratory assays and point-of-care tests. The single-use nature of the cell-free system may impose practical usability and cost constraints; however, suitable self-replicating cell-free circuits for these applications might become feasible in future. In addition, although cell-free protein synthesis using recombinant elements show good sensitivity, protein yield is low, and so more robust and standardized cell-free gene-expression systems would be needed. Naturally, many other performances and stability challenges — such as operational robustness in humid environments or in environments much hotter or colder than ambient temperature, and in the presence of environmental contaminants — would also need to be worked out before the wearable genetic circuits can be developed for eventual commercialization.

All in all, the versatility and applicability of programmable textile-embedded cell-free biosensors makes the technology particularly suitable for on-the-go detection of transmissible viruses, pesticides and other environmentally hazardous substances. It is estimated that more than 1,000 viruses with a high potential for human spill-over — including novel SARS-like coronaviruses — have been discovered[8]. Textile-integrated biosensors, if robust, accurate and inexpensive, would contribute to global warning systems for the prevention of pandemics. Beyond infectious diseases, freeze-dried cell-free technology could also be adapted for the monitoring of other human diseases via the detection of disease biomarkers in exhaled aerosols or sweat. ❐

Yuan Lu ✉

Key Laboratory of Industrial Biocatalysis, Ministry of Education, Department of Chemical Engineering, Tsinghua University, Beijing, China.

✉*e-mail: yuanlu@tsinghua.edu.cn*

Published online: 21 March 2022
https://doi.org/10.1038/s41551-022-00869-3

References

1. Karig, D. K. *Curr. Opin. Biotechnol.* **45**, 69–75 (2017).
2. Lu, Y. *Synth. Syst. Biotechnol.* **2**, 23–27 (2017).
3. Pardee, K. et al. *Cell* **167**, 248–259 (2016).
4. Nguyen, P. Q. et al. *Nat. Biotechnol.* **39**, 1366–1374 (2021).
5. Gootenberg, J. S. et al. *Science* **356**, 438–442 (2017).
6. Pardee, K. et al. *Cell* **159**, 940–954 (2014).
7. Pardee, K. et al. *Cell* **165**, 1255–1266 (2016).
8. Carroll, D. et al. *Bull. World Health Organ* **96**, 292–294 (2018).

Competing interests

The author declares no competing interests.