Cell-based biosensors for immunology, inflammation, and allergy

Maria Eugenia Inda, PhD,[a,b] Mark Mimee, PhD,[a,b] and Timothy K. Lu, MD, PhD[a,b,c] *Cambridge, Mass*

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Cell-based biosensors (CBBs) are genetically engineered living cells used to detect analytes with high sensitivity and specificity in a cost-effective and noninvasive manner. Naturally, cells sense external stimuli in their environment as a means to adapt and survive. By connecting natural transcriptional responses to expression of colorimetric, fluorescent, or bioluminescent reporter genes, CBBs can continuously sense biomolecules and report this information. Advances in synthetic biology have accelerated the development of CBBs toward clinical applications. Whole-cell biosensors have been developed by using either bacterial or mammalian cells, including laboratory strains and commensal bacteria from the human microbiota.[1,2] By rewiring signaling pathways in these natural systems, new diagnostic and therapeutic systems have been built to sense biomarkers associated with inflammatory, immunologic, and metabolic disorders, some of which have already progressed into clinical trials to gain regulatory approval.[3]

Why living cells? Using biology to sense biology captures the benefits of natural biosensors. For *robustness*, cells are considered robust to harsh environments and act as a buffering system to preserve functionality of the actual sensor components (eg, receptors and transcription factors), even in extreme conditions, such as low pH, high temperature, or osmotic stress. For *sensitivity*, biosensors are designed to detect molecules in a biologically relevant concentration range. For *specificity*, sensor components have highly specific recognition sites that detect analytes at the molecular level and can distinguish between isomers. Signal transduction is also generally orthogonal, allowing for precise distinction of similar biological signals in complex environments. For *evolvability*, sensor properties can be improved through directed evolution compared with chemical systems that are restrained to screening approaches. For *continuous sensing*, cells continuously sense their environment without the need for interrupted or periodic sampling. For *noninvasiveness*, given their size at the micron scale, CBBs can be deployed in difficult-to access environments, where they can be used to follow the progression of a disease and detect labile compounds that would otherwise be metabolized or inactivated in biological samples currently used for clinical testing, such as serum, urine, or feces.

All these qualities make CBB development an attractive avenue for next-generation diagnostic and therapeutic approaches. Personalized medicine is intended to be targeted to the individual and reduce systemic high-dose side effects. In personalized medicine, biosensors are particularly relevant for diseases in which signaling pathways are dysfunctional and can be retuned through synthetic genetic networks (ie, genetic circuits) to restore a healthy homeostatic state, as in patients with inflammatory, allergic, and immune diseases. Biosensors are uniquely suited for complex and difficult-to-access sites for surveillance and delivery of therapeutic agents (eg, in the gut; Fig 1).

Here we provide an overview of the advances in CBBs applied to immunology, inflammation, and allergy. First, we present a description of current state-of-the-art implementations of bacterial and mammalian sensors and conclude with our insights on using synthetic biology for future biomedical applications.

IMPLEMENTATIONS OF CBBs

Bacterial sensors

Natural systems that sense biomarkers of inflammation, such as nitric oxide,[4] thiosulfate,[5] and tetrathionate,[5,6] have been adapted for CBBs. For tetrathionate and nitric oxide CBBs, sensing events were recorded by genetic memory circuits, causing permanent activation of gene expression through bistable toggle switches or permanent modifications to the biosensor genome. Particularly, the tetrathionate biosensor was built in a commensal murine *Escherichia coli* strain, which is specifically suited for collecting information during passage through the gut. On excretion, data were recovered from a simple colorimetric assay directly from the stool samples and tested in both infection-induced and genetic mouse models of inflammation over the course of 6 months.[6]

From [a]the MIT Synthetic Biology Center; [b]the Research Laboratory of Electronics, Department of Electrical Engineering and Computer Science; and [c]the Department of Biological Engineering, Massachusetts Institute of Technology.

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Corresponding authors: Mark Mimee, PhD, or Timothy K. Lu, MD, PhD Massachusetts Institute of Technology, 500 Technology Square, Rm NE47-221, Cambridge, MA 02139. E-mail: mmimee@mit.edu OR: timlu@mit.edu.

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For real-time detection of compounds within the body, an ingestible micro-bio-electronic device was developed to detect bleeding during gastrointestinal transit.[7] As a proof of concept, probiotic *Escherichia coli* Nissle 1917 was engineered to bioluminesce in the presence of heme as a proxy for gastrointestinal bleeding. The CBBs were packaged in a capsule alongside miniaturized luminescence readout electronics, such that bacterial sensing events are communicated to electronics through light, and the data are wirelessly transmitted to an external device. By using this platform, gastrointestinal bleeding was successfully detected in the porcine stomach. Other inflammatory CBBs, such as thiosulfate sensors,[5] were also incorporated in the ingestible micro-bio-electronic device to demonstrate the modularity of the platform. The possibility to monitor compounds *in situ* as they are produced could aid in the elucidation of underlying mechanisms of disease and to improve disease management of chronic disorders.

Perhaps the greatest potential of CBBs is their integration in a sense-and-respond genetic circuit, such that detection of a disease biomarker can trigger therapeutic production. Phenylketonuria is a rare genetic disease that causes phenylalanine (Phe) to accumulate in the body, leading to neurotoxicity. To treat this metabolic disorder, *E coli* Nissle 1917 was engineered to produce enzymes that metabolize Phe only in anoxic conditions in the gut.[3] This strain has been shown to reduce the blood Phe concentration by 38% in treated compared with untreated mice, and, in healthy cynomolgus monkeys, the engineered bacteria prevented increases in serum Phe levels after a high-protein diet. Current clinical trials are being performed to gain regulatory approval, which, if successful, would become the first approved synthetic biology–based medical treatment. As the field of CBB develops, complex sensors will be able to integrate multiple signals in a sense-and-respond platform to noninvasively detect disease and trigger the production of therapeutics at the precise concentration necessary to restore homeostasis.

Mammalian cells

Human CBBs have been implemented to build a nonintrusive personalized allergy profiler. A synthetic mammalian designer cell was engineered to detect histamine production from basophils isolated from patients' whole-blood cell samples on allergen exposure. The biosensor, which is localized in the membrane, reports different levels of extracellular basophil-derived histamine through a redesigned synthetic signaling pathway that produces a fluorescent or secreted enzymatic output. Real-time detection of histamine allowed for scoring of a collection of potential allergens based on the magnitude of the IgE-dependent allergic reaction.[8] The system was validated on blood from donors with a clinical history of allergy and demonstrated strong concordance with the current gold standard skin prick test and comparatively greater sensitivity and a wider dynamic range.

To complete the sense-and-respond scheme, Chassin et al[9] developed a system that can sense extracellular cytokines (IL-4 and IL-13) expressed on on allergic reaction to trigger dose-dependent expression of an IgE inhibitor (designed ankyrin repeat protein [DARPin] E2_79). DARPin E2_79 is an engineered antibody mimetic protein that is highly specific for IgE and able to block formation of IgE–FcεRI receptor complexes, interfering with the onset of an allergic responses. Tested in whole-blood cell cultures, they showed that cells engineered with IL-4/IL-13 sensors can produce sufficient DARPin E2_79 to inhibit the allergic reaction. In concert with the histamine detector CBB, the cytokine sensor can be used to monitor the efficacy of the anti-allergic response. By using this system, engineered cells could be implanted in patients to continuously monitor cytokine and immunologic profiles and produce immunomodulatory therapeutics to stymie allergic responses.

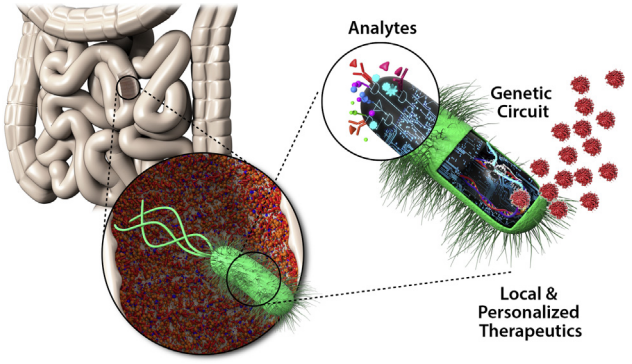

FIG 1. Bacterial CBBs allow for noninvasive molecular profiling in complex and difficult-to-access environments ideally suited for medical applications, such as continuous disease monitoring of biomarkers associated with inflammatory, immunologic, and metabolic disorders and personalized treatment *in situ*.

Human cells have been engineered to detect TNF-α and IL-22 as biomarkers of a psoriasis flare. When both signals are detected, a genetic circuit triggers expression of the therapeutic cytokines IL-4 and IL-10. These microencapsulated cells have been implanted intraperitoneally into mice topically treated with imiquimod to induce skin inflammation as a model for human psoriasis. Skin surface morphology and histochemical analysis of skin sections confirmed that healing had been achieved, demonstrating the successful expression of cytokines at physiologically relevant levels.[10]

FUTURE PERSPECTIVES

Whole-cell biosensors are still in their infancy. Currently, CBBs offer many advantages compared with conventional means of detection, such as specificity and robustness to harsh environments, among others. However, challenges in the design and implementation of CBBs, as well as in the regulation of genetically engineered organisms, must be overcome for the field to reach its full potential.

As the field matures, an array of biosensors of clinically relevant biomarkers will be available, and robust genetic circuits will be developed to integrate and process multiple signals in living cells. We envision that this technology would allow us to complete the molecular profiling needed for the personalized medicine era in a safe and noninvasive way. Living cell therapeutics could be equipped with sense-and-respond genetic circuits (with detection and response all in one) to detect a subtle homeostatic imbalance and correct it by producing the right dose of drug. The applications of this future technology are far-reaching, ranging from restoring intestinal dysbiosis to allergy relief to treatment of autoimmune diseases.

REFERENCES

1. Mimee M, Tucker AC, Voigt CA, Lu TK. Programming a human commensal bacterium, *Bacteroides thetaiotaomicron*, to sense and respond to stimuli in the murine gut microbiota. Cell Syst 2015;1:62-71.
2. Lim B, Zimmermann M, Barry NA, Goodman AL. Engineered regulatory systems modulate gene expression of human commensals in the gut. Cell 2017;169: 547-58.e15.
3. Isabella VM, Ha BN, Castillo MJ, Lubkowicz DJ, Rowe SE, Millet YA, et al. Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria. Nat Biotechnol 2018;36:857-64.
4. Archer EJ, Robinson AB, Süel GM. Engineered *E. coli* that detect and respond to gut inflammation through nitric oxide sensing. ACS Synth Biol 2012;1:451-7.
5. Daeffler KN, Galley JD, Sheth RU, Ortiz-Velez LC, Bibb CO, Shroyer NF, et al. Engineering bacterial thiosulfate and tetrathionate sensors for detecting gut inflammation. Mol Syst Biol 2017;13:923.
6. Riglar DT, Giessen TW, Baym M, Kerns SJ, Niederhuber MJ, Bronson RT, et al. Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation. Nat Biotechnol 2017;35:653-8.
7. Mimee M, Nadeau P, Hayward A, Carim S, Flanagan S, Jerger L, et al. An ingestible bacterial-electronic system to monitor gastrointestinal health. Science 2018; 360:915-8.
8. Ausländer D, Eggerschwiler B, Kemmer C, Geering B, Ausländer S, Fussenegger M. A designer cell-based histamine-specific human allergy profiler. Nat Commun 2014;5:4408.
9. Chassin H, Geering B, Schukur L, Ausländer D, Lang B, Fussenegger M. Sensing and responding to allergic response cytokines through a genetically encoded circuit. Nat Commun 2017;8:1101.
10. Schukur L, Geering B, Charpin-El Hamri G, Fussenegger M. Implantable synthetic cytokine converter cells with AND-gate logic treat experimental psoriasis. Sci Transl Med 2015;7:318ra201.