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Review article

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Microbial bioelectronic sensors for environmental monitoring

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Abstract

In a world confronting pollution across diverse environments, fast, sensitive and cost-efficient methods are required to monitor complex chemicals. In particular, microbial bioelectronic sensors can report on the presence of chemicals through electrical signals enabled by biological processes. For example, microbial bioelectronic sensors have been developed for the rapid detection of riverine toxins within minutes of contact, for selective sensing of redox-active pharmaceuticals, and for monitoring of pesticide degradation. However, transferring these laboratory-tested technologies into field-deployable products poses several challenges: sensor sensitivity, specificity, longevity and robustness need to be improved. In this Review, we discuss the design of field-deployable microbial bioelectronic sensors, including chassis selection, approaches for rewiring electron transfer, strategies to establish the cell–electrode interface and fabrication methods. Importantly, we outline key challenges and possible solutions for the application of such sensors in the real world.

Sections

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Key points

- Rapid detection of pollutants demands innovations in microbial bioelectronic sensors.
- Engineering bioelectronic sensors for environmental monitoring involves selection of a microbial chassis, rewiring of electron transfer, establishment of the cell-electrode interface and manufacture of the device.
- A microbial chassis suited for bioelectronic sensing can be found in a range of ecosystems, and electron transfer can be rewired by controlling primary metabolism or by switching electroactive components 'on' and 'off'.
- Materials can facilitate electron transfer to an electrode and enable biocontainment.
- Devices can be fabricated to amplify signals, remove environmental noise and minimize power consumption and footprint.

Introduction

Population growth, urbanization and industrialization have been driving increased pollution, causing approximately nine million deaths per year worldwide[1]. In particular, agriculture and industrial waste containing heavy metals, nitrogen, phosphorus, sulfur and organic carbon have polluted our environment (according to the Global Chemicals Outlook II: From Legacies to Innovative Solutions)[1]. Monitoring these pollutants requires detection techniques capable of on-site operation, providing results within minutes or hours, and accurately reporting even trace yet harmful concentrations. Additionally, there is a pressing need for low-cost sensors, because high costs remain a major obstacle hindering pollution monitoring in low- and middle-income countries[2], where 90% of pollution-related deaths occur[1].

Microbes can be harnessed as whole-cell biosensors to detect target analytes in complex environments. Unlike other types of biosensors that use purified enzymes, antibodies or nucleic acids as recognition elements, whole-cell biosensors employ intact microbes as sensory machines to recognize analytes, process information and transmit results[3]. Microbes naturally reside and operate in diverse locations, providing opportunities for sensing in different environments. The detection ranges of microbial recognition components often align closely with effective chemical concentrations that influence biological activities, which could be relevant to human health. Moreover, their microscopic size allows access to areas inaccessible by macroscopic probes. In addition, their self-replication and self-repair minimize the cost of manufacture and enable activity for extended periods, making whole-cell biosensors ideal tools with which to detect pollution.

Microbial whole-cell biosensors can be engineered to output various signals in response to the target analyte, including optical signals (fluorescence, chemiluminescence or absorbance)[4], altered DNA sequences (DNA memory)[5], acoustic signals[6] and electrical signals[7]. In particular, electrical signals provide advantages over other outputs, such as optical signals, which offer visual information but must be converted into electrical signals via a photodetector to be quantified by a computer[4]. In comparison, electrical signals can be captured in opaque environments without the need for additional signal conversion. DNA memory is useful for long-term information storage, but its readout is destructive[5] and must be performed ex situ. By contrast, electrical signals are more suitable for the continuous capture of transient stimuli without destroying the sensing microbes. Acoustic signals are better for locating an analyte[6], whereas electrical signals offer a faster and more direct way of detecting and quantifying an analyte. Therefore, electrical signal outputs enable high-speed and low-cost sensing for pollution monitoring.

In a microbial bioelectronic sensor, a biological sensing event is converted into an electrochemical response. This can be achieved by directly generating electrical current through a process known as extracellular electron transfer (EET)[7,8], or by indirectly altering the electrochemical profile of the surrounding environment, such as electrical impedance or potential, through the release or degradation of electroactive components[9,10]. For example, native electroactive microbes can rapidly monitor biochemical oxygen demand (BOD, a water pollution index) and assorted toxins as a proxy for cell health[11,12]. However, such sensors do not perform well in dynamic changing environments and have low sensitivity and specificity. The development of genetic tools and a deeper understanding of EET mechanisms have permitted the introduction of non-natural molecular recognition into electron-transfer pathways and allowed the construction of unresponsive strains for negative control. These approaches have enabled sensitive and specific detection of chemicals, such as carbohydrates[13], organic acids[14–17], oxyanions[18,19], endocrine disruptors[19,20], electroactive aromatic molecules[21–24], heavy metals[10,25–27] and biocides[28]. However, genetic modification is not a panacea for all difficulties faced in environmental monitoring. For example, engineered microbes may escape the detection zone, causing signal loss and environmental contamination; the deployment of microbes to non-native environments may attenuate sensor longevity; and the bulkiness and high capital cost of the electrochemical equipment may impede large-scale field applications. In addition to the sensing microbes, microbial bioelectronic sensors also require a biocompatible material to confine microbes, an electrode to receive electrical signals, a detector to read the signals and a device casing to encapsulate all components. Fine-tuning these accessory components can help to address difficulties during field deployment.

In this Review, we propose a four-step workflow for designing field-deployable microbial bioelectronic sensors (Fig. 1a): selecting a native or adaptable electroactive microbe that is suitable for the target environment; rewiring electron-transfer processes using genetic approaches to achieve selective and sensitive analyte recognition; establishing physical interfaces between cells and electrodes for efficient electron flow and signal detection; and fabricating a miniature device that encases the microbes and electronic components for use in the field. Creating such a sensor requires knowledge from many disciplines, including microbiology, synthetic biology, electrochemistry, material engineering and electrical engineering, as well as specific considerations and improvements to enable its deployment in the real world.

Microbial chassis that generate electrical signals

A microbial chassis can generate electrical signals in various ways. Microbes that evolved to perform EET can exchange electrons between cellular metabolites and extracellular electron donors or acceptors through biomolecular electron carriers[29]. Chassis that lack known EET machinery can nonetheless be engineered to heterologously express EET pathways[30,31] or to produce redox-active molecules[32–36].

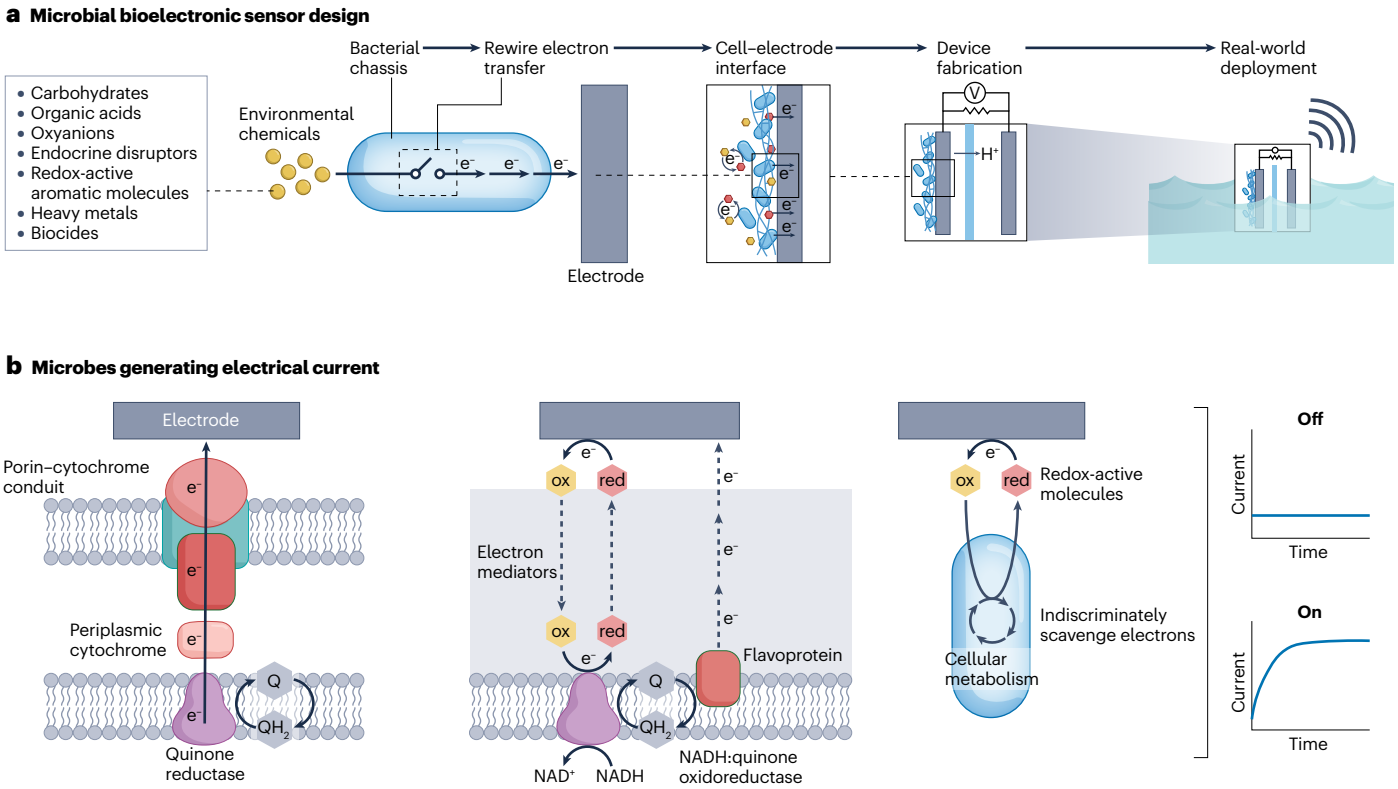

Fig. 1 | Different ways for microbes to generate electrical signals. **a**, The creation of microbial bioelectronic sensors involves selecting a microbial chassis, rewiring electron-transfer pathways for analyte recognition, interfacing or immobilizing the sensor microbe onto an electrode, and fabricating a device for real-world deployment. **b**, Microbes can produce electrical current by directly transferring electrons from the inner-membrane quinol pool to electrodes through a porin–cytochrome conduit, or by indirectly exploiting electron mediators to shuttle electrons between terminal oxidoreductases and electrodes. Alternatively, redox-active molecules can indiscriminately scavenge electrons from metabolism and transfer them to electrodes. **c**, Microbes can synthesize redox-active small molecules, whose redox activity can be monitored by electrochemical techniques, such as cyclic voltammetry, to generate electrical signals. **d**, Microbes can alter the impedance of the surrounding environment. Changed membrane permeability or impeded cell growth alter impedance in the medium, which can be monitored by electrochemical techniques. NADH, nicotinamide adenine dinucleotide; ox, oxidized; red, reduced.

Electrical signals can also be produced by unmodified, non-electroactive microbes through changes in the conductivity of the medium[10,37–39].

Native EET pathways

Microbes can natively generate electrical signals through EET using specialized biomolecular pathways to reduce electron acceptors or oxidize electron donors that cannot cross the cell envelope, such as metal minerals[29] or electrodes[8] (Fig. 1b). EET genes have been predicted in many organisms[40–42]. However, their function has been experimentally validated in only a small subset of these organisms[43]. Consequently, microbial chassis for microbial bioelectronic sensors have thus far been limited to a handful of electroactive microbes. Further characterization

of EET mechanisms in microbes from different habitats will be essential for identifying more chassis suitable for diverse target environments, given that maintaining microbial longevity in non-native environments is often challenging[44].

Shewanella oneidensis MR-1 is a leading model organism for the study of EET mechanisms. The EET pathway of *S. oneidensis* MR-1 begins with the inner-membrane tetrahaem cytochrome CymA, which transfers electrons from inner-membrane quinols to the periplasmic cytochromes small tetrahaem cytochrome (STC) and FccA[45]. Electrons are subsequently transferred across the outer membrane and onto extracellular electron acceptors by the MtrCAB complex[45]. In this complex, the transmembrane β-barrel MtrB facilitates interaction between the decahaem cytochromes MtrA (on the periplasmic side) and MtrC (on the extracellular side)[45]. This pathway supports the reduction of insoluble metals and can generate current through electrodes in bioelectrochemical systems[45]. Although *S. oneidensis* MR-1 is the best studied, many other species in the *Shewanella* genus are also capable of EET[43]. Members of the genus are found in diverse aquatic environments and have evolved the ability to grow at low temperature (<4 °C)[46,47] and high pressure (>20 MPa)[47], making them promising chassis for microbial bioelectronic sensors[48].

Evolutionarily related homologues of the Mtr EET pathway genes have been identified throughout diverse bacterial taxa[43]. Experimental validation has confirmed a role in EET for MtrCAB homologues in *Aeromonas hydrophila*[49], *Rhodopseudomonas palustris*[50] and *Vibrio natriegens*[51]. *A. hydrophila* can survive in environments with varied temperature, pH and salinity, which may make it a suitable chassis for field deployment[52]. *V. natriegens* is notable for its rapid growth (9.8-minute doubling time)[53] and the ability to take up and genomically integrate DNA from its environment[54]. *R. palustris* is a facultative phototroph that can catabolize compounds abundant in agricultural wastewater and is capable of both CO_2 fixation and N_2 fixation[55,56]. These examples illustrate the diversity of ecological niches and biological functions associated with organisms that natively express variants of the Mtr pathway of *S. oneidensis* MR-1.

Geobacter sulfurreducens PCA possesses an EET pathway that is evolutionarily distinct from, yet conceptually similar to, that of *S. oneidensis* MR-1[57]; it is another leading model system for EET. In *G. sulfurreducens*, the predominant EET flux is from the inner-membrane quinol pool through the inner-membrane proteins ImcH and CbcL[58] to the periplasmic cytochrome PpcA (and its homologues) and then to outer-membrane cytochrome–porin complexes, exemplified by OmaB/OmbB/OmcB[57]. Subsequently, anode reduction by long-range electron transfer through thick (>10 μm) electroactive biofilms relies on the outer-membrane cytochrome OmcZ[57]. The periplasmic cytochrome PpcA and the outer-membrane cytochrome OmcB have been shown to be promising candidates for establishing conditional expression control over EET[59] for biosensor development[57,60]. *G. sulfurreducens* is well suited to serve as a chassis for sensing in wastewater environments, where it readily colonizes electrodes[8]. In addition, the large currents produced by *Geobacter* biofilms may be particularly valuable for biosensing applications that demand a large signal magnitude, such as sensing in electrochemically noisy environments or self-powered microbial bioelectronic sensors[61] (Box 1). However, engineering *G. sulfurreducens* for biosensing is limited by its slow growth, its EET pathway complexity and its requirement for low oxygen concentrations[62].

The Gram-positive thermophile *Thermincola potens* JR is also capable of EET, possibly enabling sensing in high-temperature environments (about 55 °C)[63]. *T. potens* JR expresses three multihaem cytochromes on the cell surface[64], one of which can reduce an electrode and various common mediators in vitro[65]. However, not enough is yet known about how EET functions in *T. potens* JR to enable its engineering for biosensing.

Mediated electron transfer is an alternative EET strategy that exploits redox-active small molecules as mediators to shuttle electrons between terminal oxidoreductases and electron acceptors over long distances[41], allowing microbes to interact with electrode surfaces that they are not directly in contact with (Fig. 1b). For example,

Box 1 | Self-powered microbial bioelectronic sensors for low-resource settings

Microbial fuel cells (MFCs) can be used as self-powered bioelectronic sensors that do not require an external power source to function. These sensors can be energy friendly, low cost and compact, as well as suitable for long-term monitoring, remote data transmission and automation. In an MFC, electroactive microorganisms oxidize environmental substrates and generate electrons during their metabolic process. These electrons are captured by the anode, travel through an external circuit, arrive at the cathode and reduce electron acceptors, such as oxygen. The movement of electrons generates electricity that can not only be monitored as a reporting signal but also harvested to power accessory electronic components.

MFC-based bioelectronic sensors can be operated in turn-on and turn-off modes[214]. In the turn-on mode, microbes sense organic matter, which they use as an electron source to generate electricity. These MFCs can be applied to monitor biochemical oxygen demand[174], chemical oxygen demand[215] or assimilable organic carbon during water treatment[216]. In the turn-off mode, microbial electroactivity is inhibited by environmental toxins and contaminants, which subsequently decreases the power output of the MFCs. This response can be correlated with the detection of harmful substances, such as heavy metals, in polluted water[217].

The electricity produced by the MFC-based biosensor can be used to power external signalling devices or alarm systems. For example, a self-powered MFC-based sensor enables water-quality monitoring by turning on an alarm light-emitting diode (LED) and buzzer once contamination exceeds a threshold[174]. MFCs can also serve as the energy source for other sensing systems. For example, a yeast-based MFC can produce electricity to power a multicolour photodetector[218].

Self-powered microbial bioelectronic sensors are in the early stages of development and face challenges such as low power output, in particular, in miniaturized devices. This may be overcome by connecting multiple MFCs in series or parallel[219]. Alternatively, a super-capacitor can be introduced into the system to store energy. The accumulated energy can be released when adequate to support the operation of the sensor[220,221]. Perturbation in power output can also occur under fluctuating environmental conditions[214]. This can be mitigated through genetic or material modifications to ensure robust microbial behaviour under environmental pressures.

in *S. oneidensis* MR-1, flavins play an important part in shuttling electrons between the outer-membrane cytochromes and the electrode[66]. Many Gram-positive organisms possess the genes for the flavin-based EET pathway originally identified in *Listeria monocytogenes*[67]. In this pathway, electrons flow from membrane-bound reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase, Ndh2, to quinone mediators. Quinones then either carry the electrons directly to extracellular electron acceptors or indirectly deliver the electrons to extracellular flavoproteins, such as PplA[67–69]. A particularly promising chassis with this pathway is *Lactiplantibacillus plantarum*[68,69], which is ideal for deployment in food or mammalian gut environments. Another model chassis for mediated EET is *Pseudomonas aeruginosa*, which secretes the phenazines pyocyanin and phenazine-1-carboxylic acid (PCA) that support cell survival via anode reduction[70,71]. These chassis can potentially be engineered as bioelectronic sensors by controlling the pathways responsible for mediator synthesis or the proteins interacting with the mediators. Mediated EET alleviates the need for cells to contact the electrode directly, simplifying electrode and device design. However, mediators may diffuse away from the detection zone, which poses a different design challenge.

In microorganisms without a defined EET pathway, redox-active mediators, such as menadione[72], lawsone[73], methylene blue[74], ferricyanide[75], neutral red[76], thionine[77], resorufin[78] and ferrocene[79], can steal electrons from microbial metabolism and transfer them to electrodes, generating a strong electrochemical signal proportional to metabolic activity (Fig. 1b). These mediators allow chassis without specialized EET pathways to be used for microbial bioelectronic applications. However, these mediators may interrupt normal metabolic flux and may introduce unwanted changes to cellular physiology.

Heterologously expressed EET pathways

Microbes without native electroactivity can serve as chassis for bioelectronic sensors through heterologous expression of modular electroactive components, such as transmembrane electron-transfer conduits[19,30,31,80] and biosynthesized mediator molecules[81,82]. The well-characterized Mtr pathway has proved to be portable to other microbial chassis, such as *Escherichia coli*[30,80] and *Marinobacter atlanticus*[31]. For example, low-level expression of the *S. oneidensis* MR-1 *mtr* operon is sufficient to render *E. coli* electroactive[30]. However, Mtr-expressing *E. coli* produces a mere fraction of the EET flux of *S. oneidensis* MR-1[83]. The current production from *E. coli* can be improved by optimizing Mtr pathway expression levels[83], expressing additional cytochromes (CymA and STC)[83,84] and enhancing flux through the cytochrome *c* maturation pathway[85,86]. However, testing the impact of these changes on EET is labour-intensive, yielding limited exploration of the design space.

Inspiration for alternative strategies of engineering heterologous electroactivity can be found in approaches used for increasing current production in *S. oneidensis* MR-1, for example, by enhancing riboflavin biosynthesis[87] or by repressing competing electron-transfer pathways[88]. The engineering process may be further accelerated by high-throughput directed evolution, for example, to uncover structural information about MtrA in *S. oneidensis*[89]. Here, growth-based selections are applied to investigate large, pooled libraries of sequence variants, which maximizes design space coverage while minimizing time and labour[90]. These approaches to EET activity enhancement in native electroactive bacteria can also be applied to improve poor electron flux in chassis expressing heterologous EET pathways.

Non-electroactive chassis can also be designed to perform mediated EET by engineering the expression of genes encoding the biosynthetic pathway for an electron mediator that can support EET. For example, *P. aeruginosa* phenazine biosynthesis genes can be expressed in *Pseudomonas putida*, enabling *P. putida* to deliver electrons to an anode through the production of PCA and pyocyanin[81]. This engineered mediator production can be further optimized by using the operon *phzA2-G2* of *P. aeruginosa* strain PA14 to produce higher levels of PCA, which can be partially converted to pyocyanin by expression of *phzM* and *phzS*[91]. Similarly, *phzA2-G2* of *P. aeruginosa* PAO1 can be expressed in *E. coli* to enhance current production on an electrode[82].

Therefore, by heterologously expressing redox-active proteins or electron mediator synthesis pathways, non-electroactive chassis can be made electroactive, enabling the design of microbial bioelectronic sensors based on microbial chassis that naturally inhabit the field environment of interest, expanding the range of environments for deployment[31].

Altering the electrochemical profile of the microbial environment

An additional approach to generating electrical signals from non-electroactive microbes relies on the enzymatic production of redox-active molecules that can be detected by electrochemical techniques[32–36] (Fig. 1c). Non-electroactive substrates can be converted to electroactive products by conditionally expressed enzymes. For example, L-tyrosine can be converted into 3,4-dihydroxy-L-phenylalanine (L-DOPA) by tyrosinase[33], and *p*-aminophenyl-β-D-galactopyranoside (PAPG) or *p*-aminophenylphosphate (PAPP) can be converted into p-aminophenol (PAP) by bacterial β-galactosidase (β-gal)[34] or mammalian secreted alkaline phosphatase (SEAP)[92], respectively. These redox-active molecules can then be detected by cyclic voltammetry or amperometry. Unlike mediated EET, these redox-active products cannot sustain reversible redox cycles between the microbe and the electrode over time, and thus have a shorter signal duration and lower intensity. However, given that β-gal and SEAP are commonly used reporter systems, this provides a more portable approach than introducing complicated EET pathways.

Microbial behaviours, such as growth and swimming, can also produce useful electrochemical signatures (Fig. 1d). During active cell growth, metabolic activity produces charged metabolites as waste products, which increases media conductivity and lowers impedance[93]. Changes in media impedance allow electrical detection of impeded cell growth[37,38] or altered membrane permeability[39], caused by antibiotic susceptibility or cell lysis induced by growth-inhibiting genetic circuits[10]. However, the magnitude of the impedance change depends upon the organism's metabolic pathways and the composition of the solution[93], making the suitability of this approach vary across the chassis and environment. Alternatively, fluctuations in impedance caused by microbial swimming can be measured in narrow microfluidic channels[94]; however, the applicability of this strategy may be limited by the feasibility of running samples through a microfluidic system in a field environment. Notably, both impedance-based strategies enable the use of chassis without native or engineered electroactivity in bioelectronic sensing. However, these sensors are limited by non-specific responses to stimuli promoting or hindering growth or swimming.

Selecting a chassis for field deployment

Selecting a strategy for how biological signals are electrically monitored in microbial bioelectronic sensors depends on various factors (Table 1). The most important consideration is finding a chassis that can maintain its physical integrity, metabolic activity and consistent electrical signal production under the stresses of the field environment[44].

Table 1 | Chassis and strategies for engineering microbial bioelectronic sensors

Chassis name	Native environment	Notable traits	Electroactivity mechanism	Engineering strategy	Examples of detectable analytes	Refs.
Microbes with native EET pathway						
Shewanella oneidensis	Water sediments	Model EET organism	Porin–cytochrome conduit	Analytes are electron donors, acceptors or mediators	Organic acids	15,16,99
					Vitamins	21,24
				Analytes induce transcription of metabolic enzymes or EET genes	Commonly used inducer molecules	101, 103–105,108
					Heavy metals	25
					Biocides	28
Geobacter sulfurreducens	Water sediments	Obligate anaerobes, model EET organism	Porin–cytochrome conduit	Analytes are electron donors	Organic acids	14,17
				Analytes induce transcription of metabolic enzymes	Commonly used inducer molecules	100
Aliarcobacter butzleri	Water, food, animals	Pathogen	Unknown	Analytes are electron donors	Organic acids	98
Lactiplantibacillus plantarum	Plants, food, gut	GRAS	Flavoprotein, electron mediator	Analytes are electron mediators	Electroactive aromatic molecules	23
Pseudomonas aeruginosa	Water, plants, animals	Pathogen	Electron mediator	Analytes induce transcription of EET genes	Microbial quorum-sensing molecules	115
Aeromonas hydrophila	Fresh and salt water, food, animals	Survive in wide range of pH, temperature and salinity	Porin–cytochrome conduit	Potentially useful chassis for bioelectronic sensing		49
Rhodopseudomonas palustris	Fresh and salt water, soil	Photosynthesis, fixing CO_2 and N_2	Porin–cytochrome conduit			50
Vibrio natriegens	Marine environments	Fast growth, natural competence	Porin–cytochrome conduit			51
Thermincola potens JR	Anaerobic digester sludge	Thermophile	Cytochrome			64,65
Lactococcus lactis	Plants, food, gut	GRAS	Flavoprotein, electron mediator			193,194
Listeria monocytogenes	Soil, water, plants, animals, food	Pathogen	Flavoprotein, electron mediator			67
Microbes with heterologously expressed EET pathways						
Escherichia coli	Gastrointestinal tract	Model organism with large libraries of genetic parts and tools	Heterologously expressed porin–cytochrome conduit	Analytes post-translationally activate electron transfer protein	Endocrine disruptors	19
					Oxyanions	19
				Analytes induce transcription of EET genes	Commonly used inducer molecules	30,80,83–86
			Heterologously expressed electroactive biosynthesis pathway	Analytes induce transcription of biosynthesis pathway	Commonly used inducer molecules	82
Marinobacter atlanticus	Marine environment	Halotolerant	Heterologously expressed porin–cytochrome conduit	Analytes induce transcription of EET genes	Commonly used inducer molecules	31
Pseudomonas putida	Soil	Non-pathogenic	Heterologously expressed electroactive biosynthesis pathway	Potentially useful chassis for bioelectronic sensing		81,91
Microbes that non-specifically transfer electrons to mediators						
Escherichia coli	Gastrointestinal tract	Model organism with large libraries of genetic parts and tools	Indiscriminate electron scavenging	Analytes are toxic	Antibiotics	96
				Analytes are electron donors	Carbohydrates	195–197
					BOD	97

Table 1 (continued) | Chassis and strategies for engineering microbial bioelectronic sensors

Chassis name	Native environment	Notable traits	Electroactivity mechanism	Engineering strategy	Examples of detectable analytes	Refs.
Microbes that non-specifically transfer electrons to mediators (continued)						
Saccharomyces cerevisiae	Food, plants	Model eukaryotic organism with large libraries of genetic parts and tools	Indiscriminate electron scavenging	Analytes are toxic	Heavy metals	95
					Phenols	197
				Analytes are electron donors	Carbohydrates	198
					BOD	72
Shewanella oneidensis	Water sediments	Model EET organism	Indiscriminate electron scavenging	Analytes are electron mediators	Redox-active biomarkers	22
Anabaena variabilis	Water	Photosynthesis, nitrogen fixation	Indiscriminate electron scavenging	Analytes are toxic	Herbicides	199,200
Hansenula anomala	Soil, water, plants, animals	Eukaryotic	Indiscriminate electron scavenging	Analytes are electron donors	Organic acids	201
Actinobacillus succinogenes	Bovine rumen	Metabolic engineering candidate	Indiscriminate electron scavenging	Analytes are electron donors	Carbohydrates	196
Paracoccus denitrificans	Soil	Extremophilic	Indiscriminate electron scavenging	Analytes are electron donors	Organic acids	202
Enterobacter cloacae	Soil, water, food, gut	Pathogen	Electron mediator	Analytes are toxic	Heavy metals	203
Microbes that enzymatically produce electroactive molecules						
Escherichia coli	Gastrointestinal tract	Model organism with large libraries of genetic parts and tools	Conversion of tyrosine to L-DOPA by tyrosinase	Analytes induce transcription of tyrosinase and tyrosine-synthesis enzymes	Microbial quorum-sensing molecules	33
			Conversion of PAPG to PAP by β-galactosidase	Analytes induce transcription of β-galactosidase	Microbial quorum-sensing molecules	34,123
					Herbicides	35
					Oxidative stress	36
			Biosynthesis of riboflavin	Analytes induce transcription of riboflavin biosynthesis and export genes	Metal ions	26,27
Arxula adeninivorans	Soil	Eukaryotic, thermotolerant	Conversion of ascorbic acid 2-phosphate to ascorbic acid by phytase	Analytes induce transcription of phytase	Pharmaceuticals	32
Microbes that alter environmental impedance						
Escherichia coli	Gastrointestinal tract	Model organism with large libraries of genetic parts and tools	Arrested cell growth increases media impedance	Analytes impair cell growth	Antibiotics	37
			Ion leakage reduces media resistance	Analytes increase cell membrane permeability	Antibiotics	39
			Cell lysis increases media impedance	Analytes induce transcription of genes causing cell lysis	Metal ions	10
			Cell growth obstructs microfluidic channels altering channel resistance	Analytes impair cell growth	Antibiotics	38
			Microbial swimming alters media impedance	Analytes impair cell activity	Antibiotics	94
Staphylococcus aureus	Soil, water, air, human and animal surfaces and mucus	Pathogen	Arrested cell growth increases media impedance	Analytes impair cell growth	Antibiotics	37

Table 1 (continued) | Chassis and strategies for engineering microbial bioelectronic sensors

Chassis name	Native environment	Notable traits	Electroactivity mechanism	Engineering strategy	Examples of detectable analytes	Refs.
Microbes that alter environmental impedance (continued)						
Staphylococcus saprophyticus	Genitourinary tract, food, gastrointestinal tract	Pathogen	Cell growth obstructs microfluidic channels altering channel resistance	Analytes impair cell growth	Antibiotics	38
Klebsiella pneumoniae	Plants, animals	Pathogen	Cell growth obstructs microfluidic channels altering channel resistance	Analytes impair cell growth	Antibiotics	38

BOD, biochemical oxygen demand; EET, extracellular electron transfer; GRAS, generally recognized as safe; L-DOPA, 3,4-dihydroxy-L-phenylalanine; PAP, p-aminophenol; PAPG, *p*-aminophenyl-β-D-galactopyranoside.

Chassis that naturally inhabit the field environment of interest will best meet this consideration[44]. Genetic tractability is the second-most-important factor in chassis selection; genetic modification enables multiple strategies for converting a microbe into a specific, sensitive and robust biosensor. Based on the availability of environmentally durable and genetically tractable candidates (Supplementary Table 1), the chassis that can produce optimal electrical signals may be selected.

Signals derived from specific electroactive components, such as proteins or biosynthesized redox-active molecules, are preferable for sensor development, compared to electrochemical approaches that non-specifically or indirectly report on metabolism, because it is easier to distinguish the analyte-responsive signal. These components should ideally be natively expressed in the chassis, because the use of natively electroactive microbes tends to yield more robust signals than counterparts engineered to be monitored electrically[83]. Furthermore, chassis that can biosynthesize any needed mediators are better suited to field deployment because they do not require mediator resupply. All else equal, the better characterized the EET pathway in question, the easier pathway and chassis engineering. Therefore, the ideal chassis is a natively electroactive microbe indigenous to the target environment, equipped with available genetic tools and a well-characterized EET mechanism.

Rewiring electron-transfer processes for biosensing

A microbial bioelectronic sensor can be developed by linking the presence of an environmental stimulus to biological recognition and the subsequent generation of electrical signals. The overall electrical signals produced by a living cell can be an accumulation of many upstream metabolic and electron-transfer events. These metabolic and electron transfer nodes are potential regulatory targets that can be rewired for sensing. Environmental analytes can then be monitored by assessing their influence on one or more primary metabolic pathways. Additionally, individual electroactive components can be engineered to switch 'on' and 'off' in response to specific analytes.

Controlling electrical signals by modulating primary metabolism

Electroactive microbes produce current by routing electrons from cellular metabolism to electrodes. Therefore, environmental perturbations that affect microbial metabolism also influence the magnitude of current. This connection between the environment and microbial electroactivity provides opportunities for sensor development. For example, electroactive microbes capable of EET can be applied to report BOD[11] and assorted toxins[12] in wastewater or sediments (Fig. 2a). Such BOD or toxin monitors are based on the promoting or inhibiting effects of organic matter or toxins, respectively, on cellular metabolism, resulting in perturbations of the electrical outputs. Non-native electroactive microbes, such as *Saccharomyces cerevisiae*[72,95] or *E. coli*[96,97], can also be used for BOD and toxin monitoring when provided with indiscriminate electron mediators to relate metabolism to electrical outputs. Additionally, microbial EET can be used to report the presence of carbohydrates or organic acids, including glucose[13], acetate[14,17] and lactate[15,16]. These substrates provide electron sources for metabolism, resulting in an increase of current. However, these sensors rely on the global metabolic response and lack specificity; they respond to any stimulus that promotes or hinders metabolism and are incapable of distinguishing individual analytes.

Genetic engineering approaches can enhance the specificity of metabolism-based bioelectronic sensors. For example, an analyte-unresponsive null strain can be created by knocking out the responsive gene from the genome; null strain activity can then be compared to the wild-type strain to indicate the presence of the analyte (Fig. 2a). For example, in *Aliarcobacter butzleri*, knocking out the gene encoding lactate permease LctP or acetate kinase AckA allows the detection of acetate or lactate, respectively, because the respective null strain shows diminished current compared to the wild-type strain[98]. Fumarate can be sensed by constructing a null *S. oneidensis* strain lacking fumarate reductase FccA; here, the presence of fumarate leads to fluctuations in the current produced by the wild-type strain, but not by the null strain[99].

Controlling cellular metabolism also enables the detection of other types of analyte by using analyte-inducible transcriptional elements to drive the expression of an essential metabolic enzyme. For example, the growth and electrical outputs of *G. sulfurreducens* can be linked to the sensing of small molecules or ions by expressing the citrate synthase GltA, acetyl–CoA transferase Ato1 or D-lactate dehydrogenase D-Ldh under analyte-inducible promoters in strains lacking the native copy of the respective gene[100]. Similarly, an *E. coli* and *S. oneidensis* co-culture can be made to respond to environmental inducers by controlling the expression of β-galactosidase LacZ in *E. coli*, which regulates the conversion of lactose to lactate. Lactate can then be used by *S. oneidensis* to produce current[101].

Controlling electrical signals by switching electroactive components 'on' and 'off'

A robust understanding of the electron-transfer mechanism enables the construction of microbial bioelectronic sensors through regulating specific electroactive components[102], such as transmembrane

electron-transfer conduits, intracellular protein electron carriers, oxidoreductases and redox-molecule-synthesizing enzymes. Synthetic biology tools allow the manipulation of such components on transcriptional, post-transcriptional, translational or post-translational levels to link their activation to a sensing event (Fig. 2b). Unlike global regulation of electron transfer in metabolism, controlling local electron transfer mediated by specific electroactive components circumvents the need to modulate metabolic pathways, which improves cell viability during sensing.

Components of the well-studied Mtr pathway in *S. oneidensis* can be regulated for sensing. Taking advantage of the native interaction between electron mediators and the outer-membrane cytochromes MtrC and OmcA, wild-type *S. oneidensis* can be harnessed to sense riboflavin[21,24], an essential vitamin and pyocyanin[22], the biomarker of *Pseudomonas aeruginosa* infection. Mtr EET can also be transcriptionally controlled by modulating the membrane-bound *c*-type cytochrome CymA and the transmembrane conduit MtrCAB. Various transcriptional

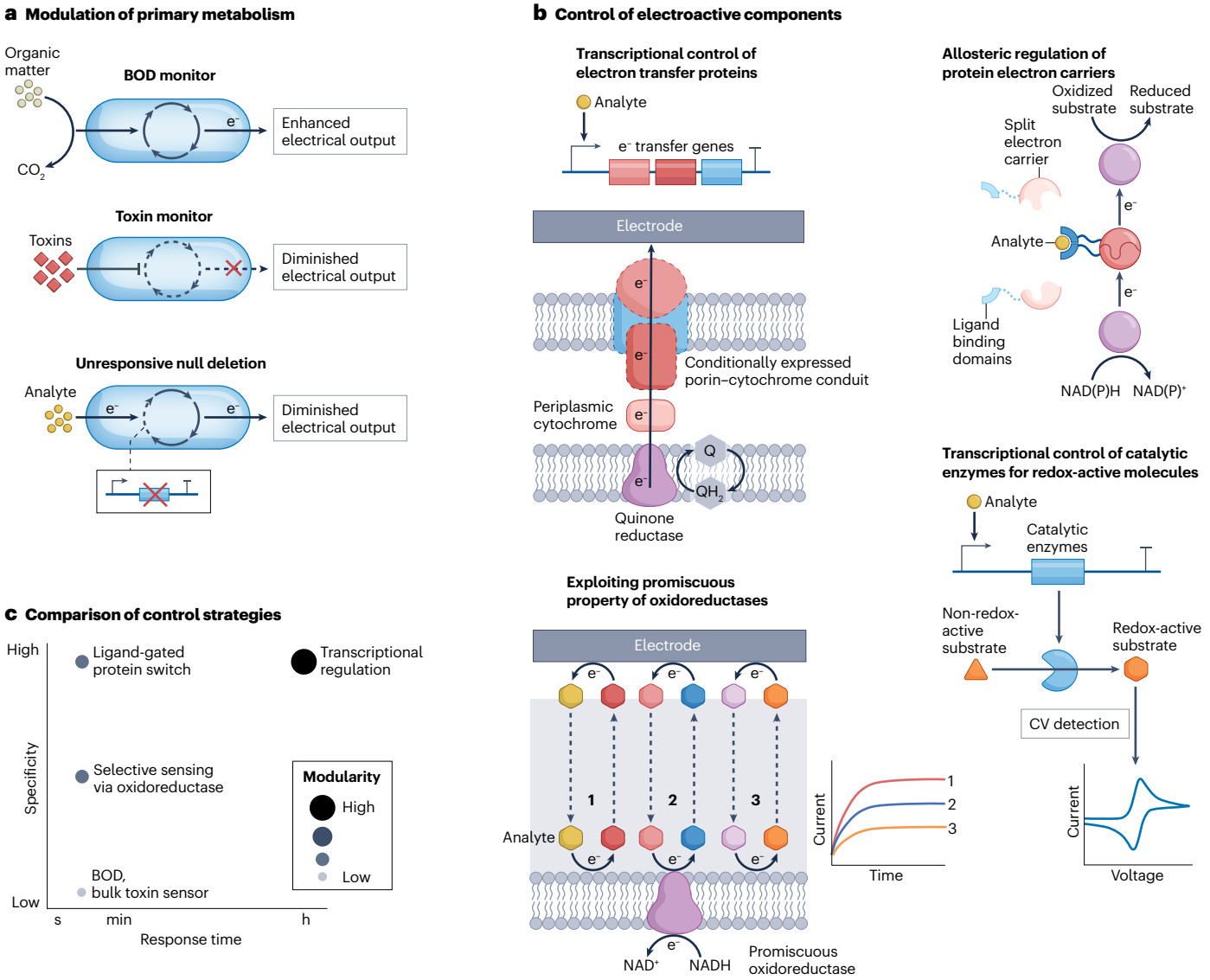

Fig. 2 | Strategies for rewiring electron transfer for bioelectronic sensing. **a**, Primary cellular metabolism can be exploited for bioelectronic sensing. Biochemical oxygen demand (BOD) and toxins can be monitored based on the promotive or inhibitive effects of organic matter or toxins, respectively, on cellular metabolism, resulting in perturbations in electrical output. Specific sensing can be achieved by comparing the electrical output between an analyte-unresponsive null strain and a wild-type strain. **b**, Individual electroactive components can be controlled for bioelectronic sensing. The expression of electron-transfer proteins can be controlled by analyte-inducible transcriptional elements. Protein electron carriers can be post-translationally manipulated through the creation of a ligand-gated protein switch responsible for the analyte. Oxidoreductases, with their promiscuous substrate-binding ability, can be used to selectively sense a group of redox-active analytes. Catalytic enzymes responsible for synthesizing redox-active molecules can be controlled by analyte-inducible transcriptional elements. **c**, Different control strategies vary in their response time, specificity and modularity. Modularity refers to the degree to which the regulatory elements or electron-transfer components can be interchanged and customized for different sensing purposes. NAD(P)H, reduced nicotinamide adenine dinucleotide (phosphate); CV, cyclic voltammetry.

regulatory elements, including inducible promoters and CRISPR interference (CRISPRi), have been applied to modulate Mtr EET in response to environmental cues, such as L-arabinose[103], rhamnose[104], trimethylamine *N*-oxide (TMAO)[105] or guide RNA (gRNA) binding to targeted genome sites[106]. Additionally, translational control machinery, such as small regulatory RNA (sRNA) molecules, can be used to tune the translation of Mtr proteins[106]. To enable pollutant sensing, the *cymA* of *S. oneidensis* can be placed under the *p*-nitrophenol inducible promoter, which responds to the degradation product of the pesticide paraoxon. Co-cultured with paraoxon-degrading *E. coli*, the engineered *S. oneidensis* system can specifically detect paraoxon and generate current[28]. Similarly, an arsenite-inducible promoter can be used to regulate *mtrB* and produce current in response to arsenite[25]. Moreover, genetic logic gates (for example, buffer, NOT, AND, OR, NOR and NAND) can be implemented to precisely control Mtr-pathway components in response to one or more chemical stimuli[107,108], enabling biological computing in electron-transfer processes.

Protein electron carriers, such as ferredoxins or flavodoxins, are another group of electroactive components that can be manipulated for sensing. These proteins are intracellular electron hubs that control electron transfer in many cellular activities, such as nutrient assimilation, energy conservation and primary metabolism[109,110]. Synthetic electron-transfer pathways can be created by coupling protein electron carriers with one or more oxidoreductases[111–113]. For example, an eight-component synthetic electron-transport chain can be constructed in the *E. coli* strain EW11 for the rapid detection of environmental contaminants[19]. This pathway links sulfur metabolism to current generation through EET. When an anion pollutant thiosulfate is present, it is converted into sulfide via an input module composed of ferredoxin–$NADP^+$ reductase, ferredoxin (Fd) and sulfite reductase. The sulfide then acts as the electron source for a coupling module containing a sulfide:quinone reductase to reduce the inner-membrane quinone pool. Finally, the electrons are transferred to the electrode via a CymA–MtrCAB output module. To diversify the sensing ability, the Fd can be transformed into a protein switch that is allosterically regulated by chemical stimuli[19,20] (Fig. 2b). Fused with oestrogen-receptor ligand-binding domains, the electron transfer of Fd is post-translationally gated by the endocrine disruptor 4-hydroxytamoxifen (4-HT)[19]. Therefore, by integrating individual electron-transfer components in a specific chassis, it is possible to de novo assemble 'living electronics' that can interface with electrodes and respond to environmental stimuli[102].

A deeper understanding of electron-transfer mechanisms driven by specific oxidoreductases has inspired new sensing strategies. Many NAD(P)H-dependent oxidoreductases are promiscuous enzymes that can catalyse redox reactions with a range of distinct but related substrates that contain similar functional groups, such as amines, alcohols or ketones[114]. This attribute can potentially be hijacked to detect a diverse set of natural or synthetic substrates and subsequently convert the detection into electrical outputs (Fig. 2b). For example, a genome-modified *L. plantarum* can sense pharmacologically relevant quinone derivatives through the Ndh2-dependent EET pathway[23]. This sensor can generate distinguishable current in response to different quinone analogues based on their differing kinetics in reacting with Ndh2. The magnitude of the current is determined by quinone physicochemical properties and can be deciphered by a multivariate model. Notably, quinone is recycled in the EET pathway for multiple rounds of electron shuttling, and thus, this sensor can intrinsically amplify signals, making its sensitivity higher than traditional analytical sensing systems, such as high-performance liquid chromatography.

Redox-active molecules can also serve as reporters for the presence of a specific analyte (Fig. 2b), and enzymes involved in the synthesis of these molecules can be controlled for sensing. For example, the phenazine synthesis *phz* operon in *P. aeruginosa* can be controlled to activate phenazine-mediated EET only when the strain senses quorum-sensing signal molecules[115]. Similarly, the flavin synthesis *rib* operon in *E. coli* can be engineered to respond to heavy metals, such as copper[27] and zinc[26]. These endogenous operons use native metabolites as substrates, and therefore, such sensors are self-sustaining and do not require an external supply of precursors. However, these pathways tend to contain multiple enzymes and are potentially subjected to complex regulation, rendering them difficult to rewire for sensing. A simpler alternative is to control a single synthesis enzyme capable of converting non-redox-active substrates into redox-active molecules. For example, the expression of tyrosinase[33] and β-galactosidase[34–36] in *E. coli* can be controlled by analyte-inducible promoters for sensing. These enzymes convert L-tyrosine or PAPG into redox-active L-DOPA or PAP, respectively, which can be detected by electrochemical techniques. However, these sensors require an exogenous supply of non-redox-active precursors, making their operation less self-sustaining, compared to endogenous redox-molecule-synthesis pathways.

Selecting a strategy to rewire electron transfer

The engineering strategy selected to rewire electron transfer substantially influences sensor performance (Fig. 2c). Analyte concentration dynamically changes in real environments, and so deployable biosensors must respond quickly within minutes or hours. Microbial bioelectronic sensors inherently meet this criterion because electron transfer is relatively fast in living organisms, with the rate constant k_0 estimated to be around 1 s^{-1} through the whole cell of *S. oneidensis*[116]. However, when genetic regulatory mechanisms such as transcriptional regulation are employed, the rate decreases owing to the involvement of gene expression (about 80 base pairs per second) and protein synthesis (about 20 amino acids per second)[117]. Consequently, the response time for these sensors typically ranges from hours[26–28,33,34,36,92,103,105,118] to days[25]. Despite the slow speed, transcriptional regulation is still valuable owing to its high specificity and modularity. Transcriptional elements previously developed for environmental and health monitoring[119–121] can be readily adapted to control electrical outputs, substantially broadening the range of detectable analytes. Synthetic biology approaches that optimize the operator, promoter and ribosome binding site[122] can also improve response time and enlarge the dynamic range.

Post-translational control of electron flux can also reduce the response time to minutes or seconds. For example, the electrical signals of electron mediators, such as flavin or quinone, detected by oxidoreductases in *S. oneidensis* or *L. plantarum*, can be observed within seconds[21,23]. Similarly, an engineered *E. coli* sensor, detecting assimilated thiosulfate or 4-HT by sulfite reductase or a ferredoxin protein switch, respectively, responds within minutes[19]. Here, detection times are probably limited only by mass transport across the solution and cell envelope, which may be overcome by introducing a permease on the cell membrane[123] to increase the substrate intake rate and ensure robust electrochemical measurements. However, oxidoreductases and protein switches are less modular than transcriptional regulation, limiting the range of detectable analytes. To address this challenge, directed evolution can be used to evolve new protein switches or oxidoreductases capable of sensing non-native or synthetic substrates.

Direct integration of regulatory elements into the genome can improve sensor stability by preventing plasmid loss and by mitigating

the burden caused by overexpression. For example, a synthetic operon consisting of an arabinose-inducible promoter controlling a hybrid *mtrFAB* complex can be inserted into the genome of *S. oneidensis* to design a sensor with higher sensitivity to a greater range of arabinose concentrations, compared to the plasmid-based counterpart[103]. Thus, careful selection of engineering strategies is crucial to improving sensor performance for real-world applications.

Materials for the cell–electrode interface and biocontainment

The development of microbial bioelectronic sensors requires a variety of material challenges to be addressed (Table 2). First, charge collection occurs at a solid electrode, which can be organic or inorganic and micro- or nanostructured. Second, the interface between the electrode and microbes governs adhesion, mediates charge transfer and can

Table 2 | Materials for electrodes, the cell–electrode interface and biocontainment

Materials	Examples		Advantages	Challenges and manufacturing techniques		Refs.
Materials for electrodes						
Carbon	Glassy carbon		Biocompatible, low-cost, stable	Certain carbon electrodes require surface treatment to improve wettability		204,205
	Carbon paper					128,206
	Carbon cloth					207
	Carbon felt					19,158
	Carbon brush					127
Metal	Gold		High conductivity, patternable	High cost; two-dimensional, vulnerable to corrosion, low biocompatibility		133,134
	Stainless steel					135
	Copper					136
	Nickel					137
Materials for cell–electrode interface						
Nanomaterial	Metallic nanoparticles	Au nanoparticles	Large surface area, high conductivity	High cost, complex fabrication process		208
		FeS_2 nanoparticles				145
		Pt nanoparticles				209
		TiO_2 or TiO_2@TiN nanoparticles				144,146
	Carbonaceous nanoparticles	Graphene				209,210
		Carbon nanotubes				147
		Carbon black				211
Polymer	Poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS)		Processible, patternable, easy integration, tunable, low cost	Conductivity depends on processing strategies and additives; can degrade or lose conductivity over time		158,159
	Polyaniline (PANI)					212
	Polypyrrole (PPy)					213
	Cationic polythiophene derivative (PMNT)					157
Composite electrode	Carbon–polymer composites		Processible, biocompatible, high conductivity	Requires additional processing and optimization		154,160
	Carbon–metal composites					139
Materials for encapsulation						
Hydrogel	Natural polysaccharides	Agarose	Biocompatible, high water content, permeable	Poor mechanical properties, low conductivity, reduced mass transport	Can be manufactured by dip coating, moulding, 3D printing, electrospinning, electrospraying or microfluidics	19,158,162
		Chitosan				161
		Alginate				19
		Agar				134
	Synthetic polymers	Polyacrylamide				162,163
		Polyvinyl alcohol				152,162
Thin polymer coating	Polydopamine		High conductivity, thin film	Very thin, insufficient for encapsulation on its own		164,165

3D, three-dimensional.

participate in redox reactions. Finally, an encapsulation layer is often required to prevent microbial release into the environment and mitigate harmful environmental effects.

Materials for electrodes

Electrodes serve not only to collect charge but also to interact with microbes. The chemistry, electronic properties and morphology of electrodes therefore influence both charge collection and microbial adhesion[124,125].

Carbon and metal are often the material of choice for microbial electrodes. Carbon-based materials, especially graphite, offer high electronic conductivity, stability, biocompatibility and cost-effectiveness[125]. Graphite electrodes are typically used in the form of rods[126], fibre brush[127], carbon paper[128] and carbon felt[19]. However, unmodified carbon mesh and carbon felt can impede microbial attachment and current generation because of their hydrophobicity. To address this problem, pre-treatment methods, including heat treatment[129], ultraviolet and ozone (O_3) treatment[130], plasma treatment[131] and chemical treatment[132], can be applied to introduce oxygen- or nitrogen-containing functional groups on carbon surfaces to polarize and activate carbon electrodes.

Noble metals, such as gold[133,134], stainless steel[135], copper[136], nickel[137] and others, are also used as electrodes in bioelectrochemical systems. The conductivity of metals is several orders of magnitude greater than that of carbon-based electrodes, thereby mitigating power-output loss. However, metal electrodes are relatively high-cost, susceptible to corrosion, and have poor affinity to microbial cells compared to carbon-based electrodes. To improve their performance, the metal surface can be coated with carbon materials, such as graphene, to protect the metal from corrosion[138]. In addition, composite carbon–metal electrodes can be fabricated to improve biocompatibility[139]. Microbes can also be engineered to express metal-binding peptides, such as gold-binding peptide[140], to enhance their affinity to metal electrodes.

Electrode morphology also affects electrode properties. Carbon-based electrodes, such as glassy carbon and metallic electrodes, are two-dimensional and non-porous and are thus not ideal for cells to attach. Three-dimensional electrodes with large pores provide more surface area, which allows better microbial access and adhesion to the electrode surfaces, enhancing the electron-transfer rate[124] (Fig. 3a). Both metal- and carbon-based electrodes can be engineered into 3D structures. For example, a 3D inverse opal indium tin oxide (IO-ITO) electrode with spherical, interconnected pores 8–10 μm in size promotes the attachment of *G. sulfurreducens*, resulting in a current density of 3 mA cm^{-2}[141]. 3D porous carbon anodes can be fabricated by 3D printing[142], where the pore size can be fine-tuned by controlling the carbonization processes, enabling a tailored 3D carbon structure for optimal bacterial growth and mass transfer.

Materials for the cell–electrode interface

The cell–electrode interface has a vital role in bridging communication between living organisms and non-living electronics. In particular, nanoparticles and organic polymers can be designed to promote cell adhesion and influence the electron-transfer rate between microbes and electrodes.

Electrode properties can be modified by using metal-based or carbon-based nanomaterials to increase electrode conductivity and the surface area for microbial attachment[143] (Fig. 3a). Nanomaterials can facilitate EET in electroactive microbes by transporting charge between the microbe and the electrode, inducing microbial nanowire formation and interacting with membrane-bound cytochromes or electron mediators. For example, TiO_2 nanoparticles can stimulate the formation of conductive nanowires and promote EET in *Geobacter*[144]. FeS_2-nanoparticle-modified reduced graphene oxide anodes can facilitate the adhesion of *Geobacter* and improve both direct or indirect electron transfer through interacting with outer-membrane *c*-type cytochromes or flavin mediators, respectively[145]. Nanoparticles can also enhance the performance of synthetic electron-transfer pathways in non-native electroactive microbes. For example, a TiO_2@TiN nanocomposite[146] can be incorporated into a carbon felt-hydrogel matrix containing an engineered *E. coli* bioelectronic sensor to improve sensor reproducibility, signal-to-noise ratio and response time[19]. Moreover, nanoparticles can create nanoscale porous structures for efficient electron transfer. For example, a 3D textile polyester cloth can be coated with carbon nanotubes to form a carbon-nanotube–textile anode with an interwoven fibre structure containing macroscale spaces (~100 μM)[147]. This fibrous and porous structure facilitates biofilm formation and enhances the electron-transfer rate from the attached microbes.

Redox-active and semiconductive polymers can also be used to enhance cell adhesion and support efficient charge transport[148,149]. Redox-active polymers are synthesized by incorporating redox-active molecules, such as osmium[150], quinone[151] or ferrocene[152], into the polymer main chain, which enables electron transfer through a hopping mechanism[149]. Applied to cell–electrode interfaces, the redox centres can extend into cell walls or membranes and mediate electron transfer from cellular redox components, such as cytochromes or oxidoreductases. For example, redox-active polymers containing quinone moieties can mediate electron transfer from Gram-negative *S. oneidensis*[153] and Gram-positive *Enterococcus faecalis*[151]. Although Gram-positive bacteria possess thick, nonconductive cell walls and rely on mediators for EET, the use of redox-active polymers can substitute the need for external electron mediators and provide opportunities to use these bacteria in bioelectronic systems.

Conductive polymers with positively charged backbones (p-type doped) — such as polyaniline (PANI)[154], polypyrrole (PPy)[155,156], polythiophene (PT)[157] and poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS)[158,159] — can bind to negatively charged bacterial outer membranes to create an interface for electron transfer[148]. In particular, PEDOT:PSS benefits from high conductivity and electrochemical stability. The monomers of these polymers can undergo polymerization in situ, forming a conductive coating surrounding the microbial cells[155,156,158]. Although monomers, such as the PEDOT precursor 3,4-ethylenedioxythiophene (EDOT), are toxic to cells, a flow-through method can be applied to reduce the cell–monomer contact time, thereby allowing in situ encapsulation of cells into the PEDOT:PSS matrix without affecting viability[158]. Alternatively, monomers can be polymerized directly on electrode surfaces to form adhesive thin films. For example, a solution-deposited thin film that comprises PEDOT:PSS, poly(2-hydroxyethylacrylate) (PHEA) and polydopamine (PDA) can be coated onto an ITO electrode[159]. The PHEA crosslinking ensures the integrity of the PEDOT:PSS film and the PDA aids in the attachment of PEDOT:PSS to ITO. This electrode remains stable for up to 12 days and shows high microbial adhesivity, leading to a 178-fold increase in current density compared to bare ITO. The incorporation of nanomaterial additives can further improve the electrochemical properties of conductive polymers[160]. For example, a composite anode of carbon nanotubes and PANI demonstrates higher conductivity than PANI alone[154].

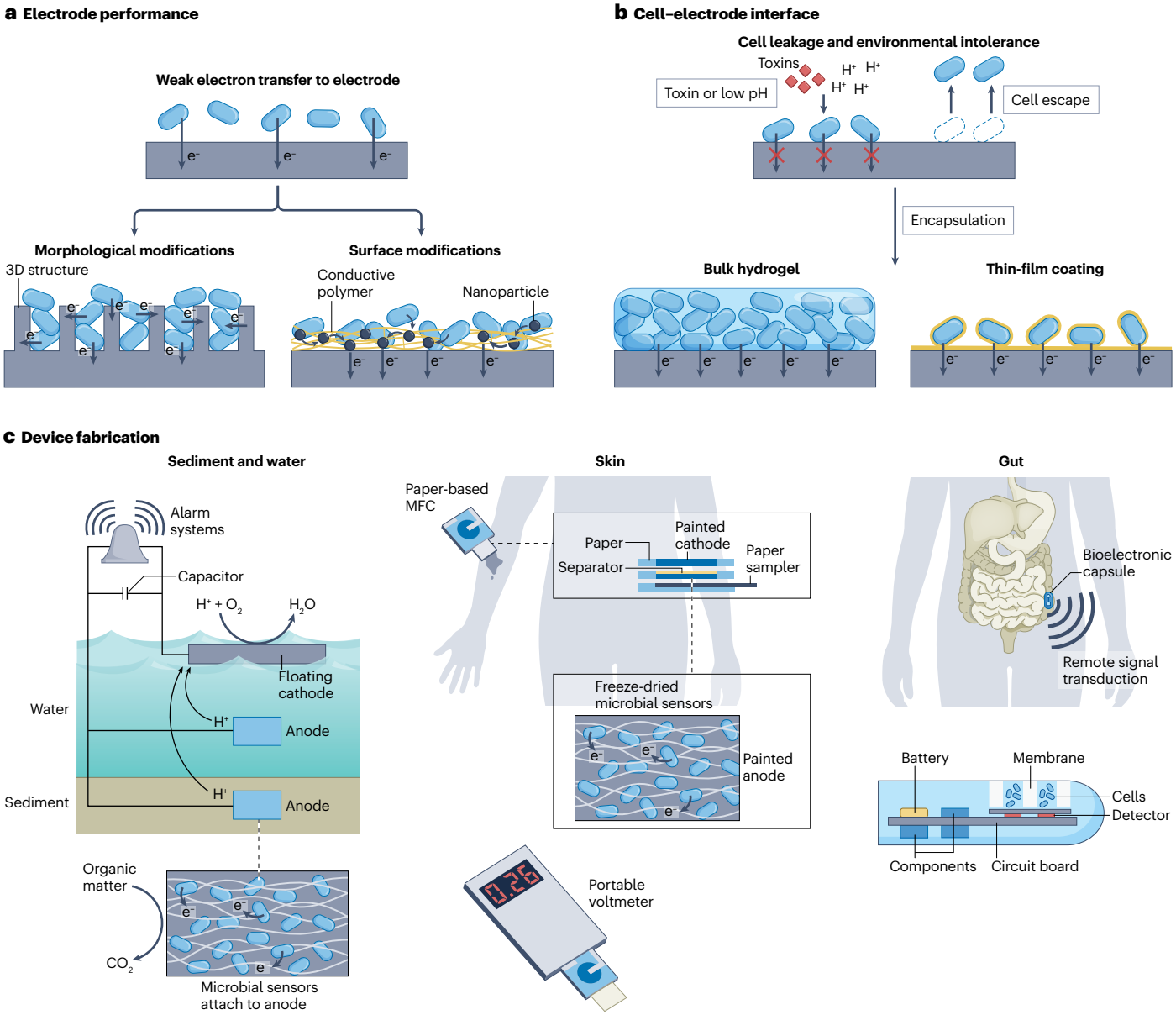

Fig. 3 | Strategies for establishing cell–electrode interfaces and manufacturing bioelectronic devices. a, The performance of electrodes can be improved by fabricating three-dimensional and porous electrodes with large surfaces for cell attachment. The conductivity and adhesivity of electrodes can be enhanced by modifying the electrode surface with conductive polymers and nanoparticles. **b**, Maintaining close contact between microbes and electrodes is essential for signal detection and biocontainment. The microbes can be immobilized on the electrodes by hydrogel encapsulation or through thin-film coating. Encapsulation also enhances microbial tolerance to environmental perturbations. **c**, Device fabrication strategies for different environmental settings. Sediment and floating microbial fuel cells (MFCs) can be created for sensing in sediment or water. The electricity generated from the MFCs can be harvested to power alarm systems. A paper-based disposable MFC is suitable for single-use detection on human skin. A miniature all-in-one bioelectronic capsule can sense, capture and wirelessly transmit the signals to an external device, allowing in situ detection in remote or inaccessible environments, such as the human gut.

These material options for electrodes and electrode modification strategies can have profound implications for bioelectronic sensing. To build miniaturized devices suitable for field use, electrodes with high conductivity, large surface area, strong adhesiveness and non-toxicity should be selected. These electrodes should ensure robust cell–electrode interaction and boost microbial electroactivity.

Materials for encapsulation

Deploying bioelectronic sensors in real-world environments demands a biocontainment strategy to prevent the escape of microbial cells. Encapsulating microbes around electrodes also facilitates signal detection and helps to resist environmental harm to the microbes. Although conductive polymers can enhance cell adhesion, they are

toxic and require harsh conditions (ultraviolet, heat) for polymerization and crosslinking, and are therefore not ideal for encapsulation. Alternatively, hydrogels and thin-layer polymers undergo gelation or polymerization in milder conditions, enabling in situ encapsulation on the electrode.

Hydrogels are ideal materials for encapsulating living cells because of their soft, wet, porous and biocompatible nature (Fig. 3b). Suitable polymers for microbial encapsulation include naturally derived polysaccharides, such as alginate[19], chitosan[161], agarose[19,158,162] and agar[134], as well as synthetic polymers, such as polyacrylamide[162,163] and polyvinyl alcohol[152,162]. These polymers are biocompatible, and hydrogels can be formed through thermal gelation or ionic or chemical crosslinking of these polymers under mild conditions within seconds to hours. Therefore, microbes can be encapsulated within hydrogel networks in situ. To render hydrogels conductive, redox-active molecules, such as riboflavin[134] and ferrocene[152], or nanoparticles, such as TiO_2@TiN[19], can be introduced into the hydrogel structure. In addition, multi-layer hydrogels can be created to increase their mechanical strength. For example, an alginate-agarose double-layer hydrogel can be applied to immobilize an engineered *E. coli* sensing strain on a carbon-felt working electrode[19], thereby improving the signal-to-noise ratio by over 30-fold, compared with non-encapsulated cells. Although it has not yet been applied in bioelectrochemical systems, *E. coli* could be encapsulated in an alginate inner core and then coated with a tough polyacrylamide shell[163] to provide biocontainment for at least 72 hours.

In addition to bulk hydrogel encapsulation, bacteria can also be encapsulated by thin polymer coatings (Fig. 3b). In particular, polydopamine (PDA) is usually used to enhance the adhesive properties of the electrode surface. For example, an electroactive microbial biofilm can be encapsulated by ~50-nm PDA films surrounding the bacterial cells[164]. This thin-film coating maintains biofilm adhesion to the electrode and protects the cells from extreme environmental conditions, such as strong acid shock. The redox-active catechol group in the PDA structure also facilitates electron transfer between the cell and the electrode. Similarly, the photosynthetic bacterium *Rhodobacter capsulatus* can be immobilized onto an anode through simultaneous polymerization of PDA, resulting in a ~5-fold enhancement in photocurrent production compared to the free cells[165]. Interestingly, PDA-based materials have also been explored as antimicrobial coatings[166].

Selecting a suitable hydrogel for a specific chassis requires understanding the gel–microbe interaction. The physicochemical properties of the hydrogel can affect colony growth and metabolic activity of the encapsulated microbes[167]. However, hydrogel crosslinking processes can be fine-tuned to optimize porosity, stiffness and elasticity to ensure microbial viability and function. Moreover, efficient processing strategies are needed for microbial encapsulation in devices. In particular, direct hydrogel printing may enable the rapid and low-cost manufacture of microbial bioelectronic devices. For example, 3D printing has been employed to create a living tattoo for sensing on the human skin[168]. This tattoo is composed of a hydrogel that encapsulates genetically engineered *E. coli* that can sense chemicals on the skin and produce fluorescent outputs. Similar approaches may offer low-cost strategies for the fabrication of microbial bioelectronic devices but have yet to be explored.

Devices for deployment in the real world

Signal amplification and processing in the device

The typical information flow of a bioelectronic sensor device includes signal recognition, transduction, amplification and processing. Engineered microbial sensors recognize the analyte and electrodes transduce the signals; however, components for signal amplification and processing are also needed to improve sensor sensitivity and accuracy.

Sensing in real-world environments confronts multiplex interfering signals, which may be addressed by signal-amplification processes to improve the signal-to-noise ratio. In particular, organic electrochemical transistors (OECTs) can amplify ionic and/or electrical signals[169]. An OECT consists of a gate electrode, an electrolyte solution and a semiconductive polymer channel bridging source and drain electrodes. By applying a gate voltage, ions from the electrolyte are injected into the channel, resulting in doping (or de-doping) of the semiconductive polymer. This process leads to large changes in polymer conductivity, thereby affecting the source–drain current. Doping changes take place throughout the entire volume of the channel, and so OECTs can convert small voltage signals at the gate electrode into considerable changes in the drain current[169], making them effective amplifiers. In addition, OECTs function at low voltages, can be fabricated on stretchable substrates[170] and enable miniaturization of devices. Moreover, they can perform in complex environments, such as body fluids[170,171], making them suitable for bioelectronic sensors. For example, *S. oneidensis* MR-1 can be deposited onto a 0.25-mm^2 PEDOT:PSS-PVA gate electrode to create microbial OECTs for signal amplification[172], resulting in an EET response to lactate an order of magnitude faster than measurements obtained by classical electrochemical techniques such as chronoamperometry.

Signal amplification can also be achieved through redox cycling. For example, a gold electrode coated with a catechol–chitosan redox capacitor can amplify the detection of the redox-active bacterial metabolite pyocyanin[173]. Here, the electrochemical detection of pyocyanin is amplified through redox cycling reactions of the capacitor, including the acceptance, storage and donation of electrons to and from pyocyanin, resulting in a substantial reduction of the detection limit for pyocyanin from 7 μM to 50 nM, compared to a bare gold electrode.

Signal processing requires filtering out noise triggered by environmental or biological fluctuations to isolate the true positive signals. The presence of living cells in microbial bioelectronic sensors makes these sensors especially prone to noise because microbes dynamically respond to environmental temperature, pH, nutrients or toxins in ways that can perturb the true signals[99]. To address this challenge, variants of the sensing elements could be designed that are unresponsive to the stimulus of interest, for example, through the deletion of the responsive gene, by designing an inactive mutant of a transcriptionally controlled EET protein or by engineering a ligand-insensitive mutant of a ligand-binding protein. Cells that harbour these unresponsive elements can be deployed alongside fully functional sensors. Perturbations in the signal of the stimulus-responsive strain that are absent in the signal of the unresponsive null strain can then be regarded as true positives[19,99]. For example, in fumarate sensing, a current ratio can be calculated using both the fumarate-responsive (wild-type) strains and the fumarate-null (Δ*fccA*) strains of *S. oneidensis*, allowing the isolation of the signal of fumarate injection from the noise caused by environmental fluctuations, such as the variation of temperature[99].

Device fabrication

Real-world biosensor devices are expected to be miniature, portable, energy-efficient, low-cost and capable of collecting and transmitting data on-site. Devices should also be robust enough to operate in dynamic real-world environments, such as riverine water, sediment and

the animal gastrointestinal tract. Strategies for fabricating microbial bioelectronic devices vary depending on environmental settings and intended applications.

Sensing in open environments, such as water or sediment, does not strictly constrain the size of the device. Thus, large devices, such as floating[174–176] or sediment[177–179] microbial fuel cell (MFC)-based sensors, can be constructed and settled on site, serving as monitoring stations (Fig. 3c). The MFC-based sensors can be connected to external equipment, such as a voltmeter or an alarm system, for data acquisition and transmission. These devices typically contain an anode with immobilized microbes immersed in sediment or water for sensing, and a separate cathode floating on the air surface or in the overlying water to transfer electrons to the atmospheric oxygen. An external load, such as a resistor or a capacitor, is typically used between the anode and cathode to control electron flow. The electrical energy generated by the MFC-based sensors can also be harvested by the external capacitor to power alarm systems (Box 1). For example, a self-powered floating MFC-based sensor enables urine detection in freshwater[174]. When the urine level exceeds the lower threshold, the electricity produced by the sensor turns on a light-emitting diode (LED) or an alarm buzzer to report urine contamination. Notably, this device can continuously monitor the water quality for up to months.

Miniature and disposable devices are suitable for single use or under constrained conditions, and may be applied for rapid water monitoring or point-of-care testing without the need for a highly trained operator. Compact MFC-based biosensors can be made using paper or silicon-based materials. Paper-based sensors use filter or chromatography papers as the foundation and are printed or painted with conductive ink as electrodes[16,180–184]. Sensor microbes can be immobilized on the paper fibre by air drying[180], freeze-drying[16,182] or hydrogel coating[183,184]. Paper-based microbial bioelectronic sensors are portable, disposable and low-cost, and thus with potential for commercialization. For example, a ~2 cm × 2 cm paper-based sensor containing *S. oneidensis* can be applied to human skin to detect lactate in sweat[16] (Fig. 3c). The voltammetric signal of the sensor can be detected on-site by a portable voltmeter with a digital display. For safe disposal to prevent material and microorganism contamination, the paper sensors can be burned with a flame[185]. Despite the weak durability of paper, this type of sensor has demonstrated its ability for long-term storage[182] and continuous sensing[183]. However, the fabrication process of

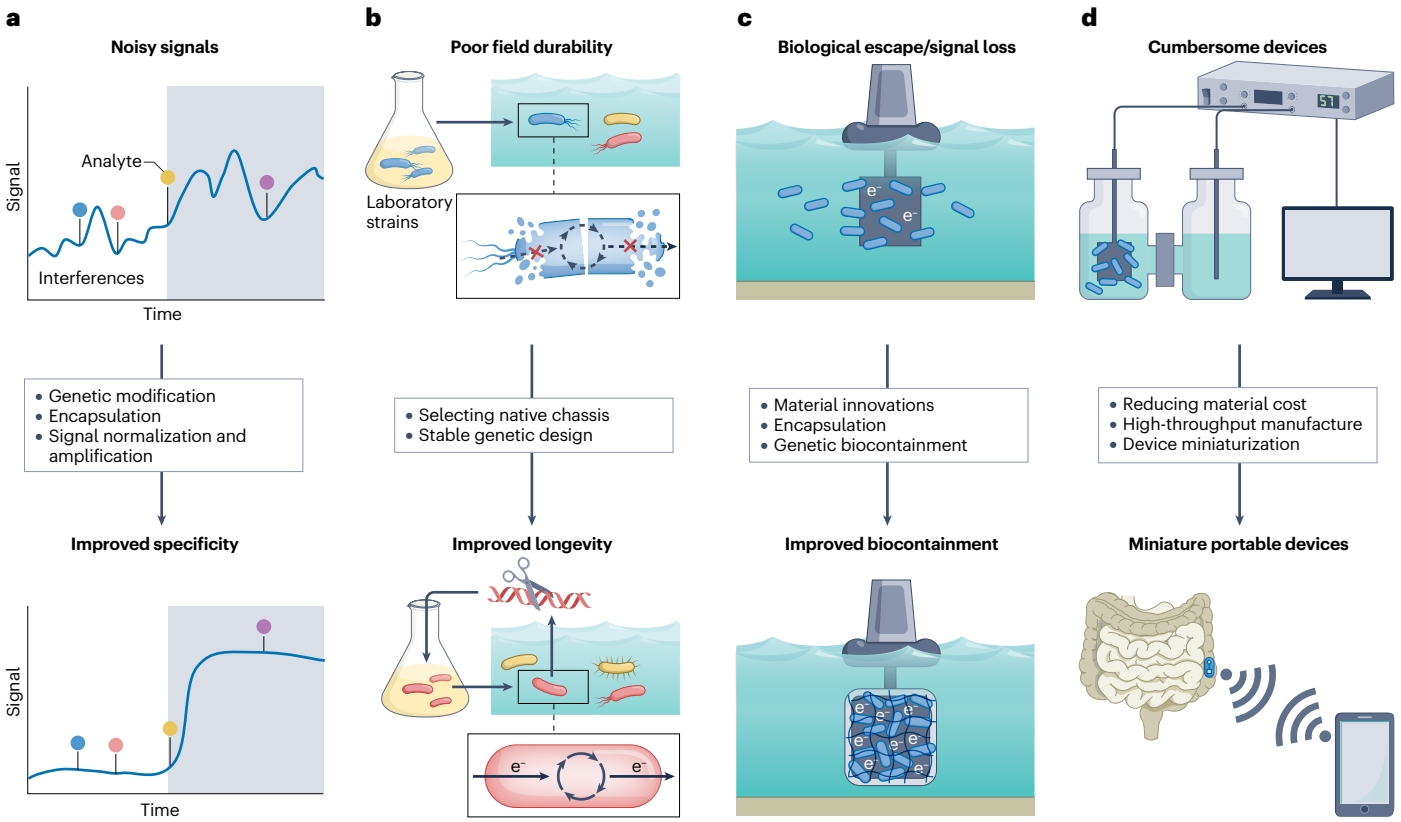

Fig. 4 | Challenges and future directions for engineering microbial bioelectronic sensors. a, Microbial bioelectronic sensors may encounter signal fluctuations and low signal-to-noise ratios in field environments. Genetic circuits can be optimized to enhance specificity, sensitivity and speed. Signal detection can be improved by encapsulating microbes on the electrodes or through signal-normalization and -amplification processes. **b**, To extend the longevity of microbial bioelectronic sensors, electroactive chassis native to the environment of interest should be selected. In addition, stable genetic design through integration of genetic circuits into the chromosome may extend sensor longevity. **c**, Electrical signals may be attenuated owing to poor electrode properties or cell escape. Innovations in materials for electrodes and surface modification may increase sensor durability and electrochemical performance. Robust biocontainment achieved by encapsulation or genetic design can prevent potential environmental contamination caused by cell leakage. **d**, The fabrication process is desired to be low-cost and scalable. Device miniaturization and the development of high-throughput fabrication techniques will reduce material consumption and accelerate the development of field-deployable sensors.

paper-based sensors may cause variations in paper size, geometry, electrode property and bacteria load, influencing the reproducibility of detection. In comparison, silicon-based devices show improved durability and less variation in fabrication. Silicon can be forged into a solid skeleton to hold electrodes and microbes[186]. In addition, silicon can act as a protective shell for the components[99]. However, owing to their complex fabrication, silicon-based devices are more costly than paper-based devices.

Such devices rely on external detectors for data acquisition, and interfacing with such equipment is not possible in certain environments, such as the human gut or deep sea. Sensing in these environments requires an all-in-one device that consists of sensor microbes, electrodes, detectors and wireless signal transducers for sending data to a remote device. Although such a device has not yet been specifically designed for microbial bioelectronic sensors, examples exist in other types of whole-cell biosensors. For example, a bacterial–electronic capsule with luminescence readout can operate wirelessly in the porcine intestine[187] (Fig. 3c). Of note, the capsule size can be reduced to 1.4 cm^2 without affecting sensor performance[188]. Similar all-in-one capsules could also be devised for electrical readout by combining an MFC-based sensor with a miniaturized voltmeter or an ammeter. Owing to its self-powering capacity, the resulting MFC-based bioelectronic capsule could be energy efficient and compact in size.

Outlook

Microbial bioelectronic sensors represent the convergence of whole-cell and electrochemical biosensors. The development of synthetic biology tools in diverse electroactive bacteria found in different environments, along with innovations in material and device design, will facilitate the transition of these sensors from the laboratory to the field. However, this transition demands fine-tuning of sensor specificity, sensitivity, longevity, robustness and fabrication processes.

To improve specificity and sensitivity (Fig. 4a), the sequence of genetic parts, including analyte-sensing proteins, reporter proteins and non-coding regulatory sequences, can be tuned to optimize performance[120,189]. To fully leverage the inherent speed of electron transfer, post-translational regulatory elements can be used to modulate the electrochemical signature without requiring transcription or translation[19]. The signal noise derived from environmental or biological variations can be mitigated by maintaining cell–electrode contact[158], by creating a stimulus-unresponsive null strain alongside the stimulus-responsive sensing strain[99] and by employing signal amplification techniques, such as redox capacitors[173] or OECTs[169].

The lifetime of a microbial bioelectronic sensor is crucial to its economic viability (Fig. 4b). Sensor longevity can be improved in non-optimal field conditions by selecting a chassis from the native environment. Electroactive microbial communities in their native environment can power bioelectronic devices for several years, as demonstrated in benthic MFCs[190] and BOD sensors[191]. With more electroactive microbes being discovered across distinct ecosystems, such as soils, sediments, marine environments, hydrothermal systems and the human gut[40], bioelectronic sensors can be designed for these different environments. Furthermore, genetic tools can be adapted to rewire electron-transfer pathways in these non-model electroactive microbes to achieve highly specific sensing. The stability of genetic circuits can be improved through chromosomal integration. However, microbes engineered for sensing need further characterization in field environments to assess the performance longevity of the engineered biological components.

The sensor device should maintain its mechanical properties and biocontainment for robust and safe deployment in the field environment (Fig. 4c). The development of new materials for electrodes and interfaces, such as redox-active polymers and composite electrodes, may improve the transport of charges and increase EET signals. Such materials may also reduce the fabrication cost of microbial-based devices, for example, through rapid additive manufacturing with in situ crosslinking to produce encapsulated and immobilized electroactive bacterial films. These material-processing strategies can be used in tandem with synthetic biology techniques, such as synthetic auxotrophy or kill switches, to prevent cell growth outside the device[192].

Microbial bioelectronic sensors also need to fit cost constraints for certain applications (Fig. 4d). Although such sensors do not require specialized equipment beyond simple electronics[99], materials costs can be prohibitive. Miniaturization of devices, in conjunction with high-throughput and low-cost manufacturing techniques, will decrease material consumption. Moreover, the need for expensive materials may be reduced by improving the signal strength and biosynthesis of electron mediators.

Although microbial bioelectronic sensors have achieved promising results in the laboratory[19,23,28], they need to be tested in the field environment to validate their practical usefulness. In-field testing will also provide invaluable insights for sensor optimization and unveil new principles for sensor design. The multidisciplinary nature of microbial bioelectronic sensor development necessitates collaboration with experts from complementary disciplines. Further innovation in stable genetic design, reliable biocontainment, durable materials development and low-cost electronics fabrication will further accelerate the deployment of microbial bioelectronic sensors. By integrating these aspects, microbial bioelectronic sensors are poised to play a vital part in monitoring environmental hazards and safeguarding the planet.

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Author contributions

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Competing interests

The authors declare no competing interests.

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