Review

Intrinsically Conductive Microbial Nanowires for 'Green' Electronics with Novel Functions

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Intrinsically conductive protein nanowires, microbially produced from inexpensive, renewable feedstocks, are a sustainable alternative to traditional nanowire electronic materials, which require high energy inputs and hazardous conditions/chemicals for fabrication and can be highly toxic. Pilin-based nanowires can be tailored for specific functions via the design of synthetic pilin genes to tune wire conductivity or introduce novel functionalities. Other microbially produced nanowire options for electronics may include cytochrome wires, curli fibers, and the conductive fibers of cable bacteria. Proof-of-concept protein nanowire electronics that have been successfully demonstrated include biomedical sensors, neuromorphic devices, and a device that generates electricity from ambient humidity. Further development of applications will require interdisciplinary teams of engineers, biophysicists, and synthetic biologists.

'Green' Electronics for a Sustainable Internet of Things

Traditional electronic materials are a boon to society, but they come at high, often hidden, costs. These can include high energy requirements for mining materials and/or component synthesis, as well as negative environmental impacts and treatment costs associated with harsh chemicals required for synthesis or toxic components in the final product [1,2]. Technical challenges and the high cost of facilities for fabricating increasingly smaller nanoscale electronic components are also becoming limiting [3].

How can the burgeoning electronics-based culture and emerging **internet of things** (see Glossary) be developed sustainably? 'Green' electronic materials sustainably produced from organic materials are an attractive possibility, especially when these materials are assembled by living organisms or are capable of *in vitro* self-assembly [1,3]. Electronic materials composed of amino acids are particularly good candidates, not only because they are produced from renewable materials but also because peptide-based electronics may provide better electronic interfaces between living systems and traditional electronics than non-biological materials [4].

Nanowires and carbon nanotubes are an important class of electronic materials [5,6]. They enable miniaturization and high-density integration of components as well as the construction of flexible electronics. Their ultrathin diameter is particularly attractive for establishing electronic connections with living cells and conveys sensing responses not found in materials with larger diameters [5,6]. However, traditional nanowires/nanotubes are not sustainably produced. For example, silicon nanowires, the most intensively investigated nanowire material, are fabricated by vaporizing the highly toxic reagents silane, diborane, and phosphine at high temperatures (>400°C), a substantial energy investment [7]. High temperatures (>500°C) are also required for the production of carbon nanotubes [8]. This process yields heterogeneous mixtures of carbon nanotubes with different properties, requiring additional processing to obtain a uniform product. Furthermore, carbon nanotubes have been placed on the SIN (substitute it now) list due to concerns about carcinogenicity, reproductive toxicity, and persistence in the environment [9].

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Microbially produced, electrically conductive, protein nanowires (i.e., **microbial nanowires**), which were discovered during basic research in the field of **electromicrobiology** (Box 1), are a potential sustainable alternative to more traditional nanowire electronic materials. Microbes fabricate these wires from renewable feedstocks with very low energy inputs and without harsh chemicals. The final product is free of toxic components and biodegradable, yet robust enough for many electronics fabrication methods and long-term function. Furthermore, microbial nanowires can offer possibilities for device fabrication, functionalization, and biocompatibility not found in other non-biological nanowire materials [2,10–14]. The purpose of this review is to summarize the known types of intrinsically conductive microbial nanowires, assess *in vivo* versus *in vitro* fabrication strategies, and provide examples of novel electronic devices employing microbial nanowires.

Pilin-Based Nanowires

A diversity of electrically conductive pilin-based protein nanowires (Figure 1), assembled *in vivo* from native type IV pilins, homologous archaeal proteins, or synthetic pilins, have been described [15]. Depending on the pilin monomer, these electrically conductive pilin-based nanowires range in diameter from approximately 3 to 10 nm (Box 2). Pilin-based wires are conductive in the absence of native metal cofactors or added metals. When still attached to cells, pilin-based wires are typically referred to as **electrically conductive pili (e-pili)** to denote their biological function [16]. E-pili have independently evolved multiple times during microbial evolution [15,17–20]. Most studied are the e-pili of *Geobacter sulfurreducens* [21] and *Geobacter metallireducens* [22], which play an important biological role in anaerobic respiration [15,23,24]. E-pili function as conduits for long-range (micrometers) electron transport to extracellular terminal electron acceptors such as Fe(III) oxides or methane-producing microorganisms. The physiology of some of the other microorganisms known to produce e-pili suggests that their e-pili have a similar extracellular electron transfer function [17,18,20]. However, e-pili may also facilitate intercellular communication or provide information about surfaces contacted [16,24].

Box 1. Electronics Inspired by Electromicrobiology

Electromicrobiology [79] is likely to continue to serve as a source of inspiration for electronics innovation. As Albert Szent-Györgyi noted, 'Life is nothing but an electron looking for a place to rest.' Thus, microbes have had billions of years to become master electricians, evolving highly effective strategies for dealing with electrons.

The term 'electromicrobiology' has only come into fashion in this century, following the discovery that microorganisms can directly exchange electrons with electrodes [80–82]. However, the possibility of microbial electrical interactions with extracellular minerals has been known since the 19th century [83]. In addition to protein nanowires, important extracellular electrical connections include *c*-type cytochromes embedded in the outer cell membrane [84] and possibly other proteins with metal-containing cofactors [85].

Intracellular *c*-type cytochromes can function as in-cell capacitors [86]. Cells gain energy as electrons derived from organic matter oxidation are shunted through typical electron transport chains that generate the proton-motive force necessary for ATP generation, but instead of electron transfer to traditional terminal electron acceptors, electrons are transferred to cytochromes, where they are stored until electron acceptors become available. When electron acceptors are encountered, the electrons stored in the cytochromes are discharged. This in-cell capacitor function can perform as a supercapacitor material that might be useful in energy storage devices [87]. Highly cohesive biofilms may serve as a 'living composite' material for this type of capacitor [88].

Microorganisms capable of electrically interfacing with electrodes function as living electronic devices that have potential applications in bioremediation of organic and metal contaminants, the production of organic commodities from carbon dioxide, and as biological sensors with direct electrical outputs [89]. These applications are an important driver for further elucidating the mechanisms of microbial extracellular electron exchange. Other motivations include the biogeochemical importance of extracellular electron transfer to insoluble minerals in soils and sediments [90], as well as the impact of direct interspecies electron transfer on the functioning of anaerobic digesters converting organic wastes to methane fuel and wetlands contributing to atmospheric methane [91]. It seems likely that these basic electromicrobiology studies will also continue to reveal novel proteins that may become useful materials for the fabrication of sustainable electronic devices.

Glossary

Curli fibers: protein filaments composed of the monomer CsgA, which self-assembles into filaments following excretion to the outside of the cell.

Cytochrome wires: electrically conductive filaments composed of multiheme *c*-type cytochromes with yet to be determined biological function.

Electrically conductive pili (e-pili): cell filaments biologically assembled from pilin monomers that are sufficiently conductive to facilitate long-range extracellular electron transfer for anaerobic respiration.

Electromicrobiology: the study of microorganisms that electrically interact with their extracellular environment either in natural settings or engineered living electronic devices.

Hydrovoltaic technologies: strategies to generate electricity from ambient moisture sources.

Internet of things: electronic devices for sensing and computing that are imbedded in everyday objects and the body and are interconnected via the internet.

Microbial nanowires: shorthand for microbially produced electrically conductive protein filaments, typically applied to the nanofilaments after they are harvested from the cells.

Memristor: a portmanteau of memory resistor, a device in which resistance can be modulated by applied voltage to store variable memory states.

Nanowires: material with a lateral dimension measured in nanometers and a substantially longer longitudinal dimension.
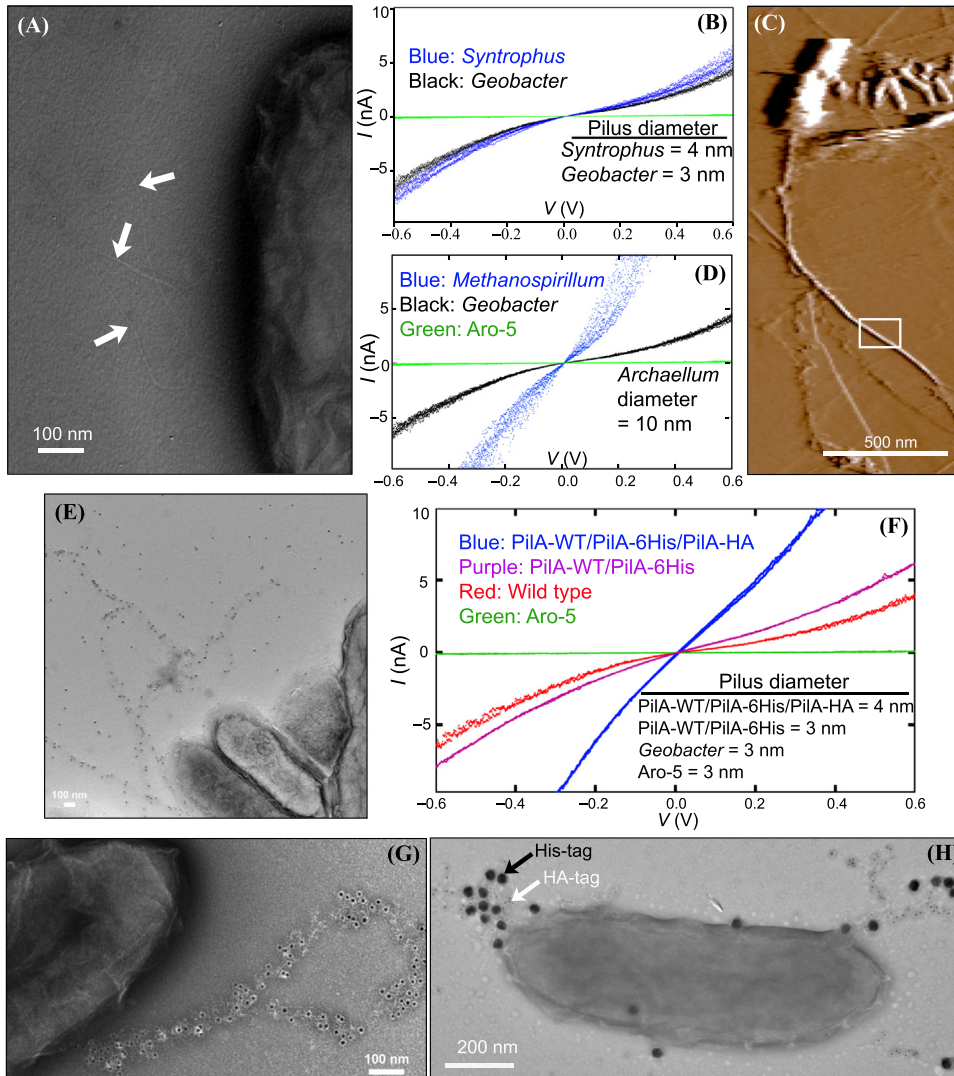
Figure 1. Examples of Pilin-Based Wires with Intrinsic Conductivity. (A) Transmission electron micrograph (TEM) of wires emanating from *Syntrophus aciditrophicus* (white arrows). (B) Conductance of individual *S. aciditrophicus* wires versus *Geobacter sulfurreducens* wires. (C) Atomic force microscope image of *Methanospirillum hungatei* with archaellum, composed of a pilin homolog known as archaellin. (D) Conductance of the archaellum in the region outlined with the white box shown in C. (EG, and H) TEMs of immunogold labeling of wires emanating from *G. sulfurreducens* expressing a synthetic pilin gene containing (E,G) a His tag or (H) separate synthetic pilin genes with either a His tag or a hemagglutinin (HA) tag, with the His tag labeled with 10-nm gold particles and HA tag labeled with 40-nm gold particles. (F) Conductance of individual synthetic wires expressed in *G. sulfurreducens* with a combination of PilA monomers that were either wild type (PilA-WT), included a His-tag (PilA-His), or included a HA-tag (PilA-HA). Adapted, with permission, from [20] (A and B), [19] (C and D), and [10] (E–H). Abbreviations: WT, wild type.

Multiple lines of evidence suggest that *G. sulfurreducens* e-pili are assembled from a 61–amino acid pilin monomer [23], as confirmed by the heterologous expression of the *G. sulfurreducens* e-pili in *Pseudomonas aeruginosa* [25] and *Escherichia coli* [11]. In some strains of *G. sulfurreducens*, e-pili are the only filaments observed emanating from cells [10] (Figure 1) or in purified filament preparations [13,26,27].

Box 2. One Size Does Not Fit All

As the study of native intrinsically conductive protein nanowires has grown beyond *Geobacter* species (Table I), the options for sustainably produced nanowire materials has been steadily increasing. For example, the pilin-based nanowires naturally available in the microbial world offer a range of diameters, conductivities, and possibilities for structural modification.

Analysis of the genomes of isolated microbes, as well as metagenomic data, suggests that an enormous number of yet to be characterized, intrinsically conductive pilin-based wires are available in the microbial world [15,18,20,92]. When these possibilities are coupled with the potential for genetically tuning conductivity and function through the design of synthetic pilins, the toolbox of protein nanowires is further expanded. It is likely that additional cytochrome-based nanowires will be discovered, increasing their potential for application in electronic devices. More information on the composition of the conductive fibers of cable bacteria could lead to the design of materials that can be readily expressed in an easy-to-culture chassis, such as *E. coli*, or assembled *in vitro*.

Table I. Basic Toolbox of Characterized Microbially Produced Nanowires with Intrinsic Conductivity

Protein nanowire source	Diameter	Conductivity of individual wire[a]	Expression in *Escherichia coli*	Genetically tunes conductivity	Genetic surface functionalization	Sensor response
Pilin-based						
Geobacter sulfurreducens	3 nm	51 mS/cm	Yes	Yes	Yes	Yes
Geobacter metallireducens	3 nm	277 S/cm	Likely[b]	Likely	Likely	Likely
Syntrophus aciditrophicus	4 nm	~ *G. sulfurreducens*[c]	Likely	Likely	Likely	Likely
Methanospirillum hungatei	10 nm	> *G. sulfurreducens*[c]	Likely	Likely	Likely	Likely
OmcS cytochrome	4 nm	30 mS/cm	Unlikely[d]	Unlikely	?[e]	?
OmcZ cytochrome	2.5 nm	12 S/cm	Unlikely	Unlikely	?	?
Cable bacterium Individual fiber	60 nm	10 S/cm	Very unlikely[d]	Very unlikely	Very unlikely	?

[a]At pH 7
[b]Possibilities for expression, tuning conductivity and function, and sensor response of pilin-based wires based on extrapolation from *G. sulfurreducens* pilin-based wires.
[c]Estimated relative conductivities based on measured differences in reported conductances.
[d]Low probability due to lack of proper systems for expression in *E. coli*.
[e](?)Designates unevaluated possibilities, with no precedent for making predictions.

The native pilin-based wires of *G. sulfurreducens* cells have a diameter of 3 nm [11,21,23,25]. Although e-pili may be 10–20 µm in length, harvested pilin-based wires are typically no longer than several micrometers due to breakage during processing. The conductivity along the length of individual pilin-based wires harvested from *G. sulfurreducens* has been reported as 51 mS/cm at pH 7 (188 mS/cm, pH 2; 40 µS/cm, pH 10.5) in one study [28] and has been estimated to be 1.4–4.3 S/cm in another study [27]. Nanowires composed of the pilin of the closely related *G. metallireducens* are more conductive (277 S/cm) [22], which has been attributed to the higher abundance of aromatic amino acids in the *G. metallireducens* pilin (see discussion on aromatic amino acids later).

Detailed investigations of the conductivity of individual pilin-based wires from other microorganisms have not been reported. However, evaluation of individual pilin-based wires from the bacterium *Syntrophus aciditrophicus* [20] and the archaeon *Methanospirillum hungatei* [19] yielded conductances slightly higher than those of *G. sulfurreducens* wires examined with the same method. Thin films of wires heterologously expressed in *G. sulfurreducens* from pilin genes from the bacteria *Flexistipes sinusarabici*, *Calditerrivibrio nitroreducens*, and *Desulfurivibrio alkaliphilus* also had conductances comparable with those of thin films of wild-type *G. sulfurreducens* wires [18]. Notably, some of the monomers of these pilin-based wires were much larger than the *G. sulfurreducens* pilin (number of amino acids: *C. nitroreducens*, 119; *D. alkaliphilus*, 182; *S. aciditrophicus*, 110).

Multiple lines of evidence suggest that the abundance and placement of aromatic amino acids in pilin-based wires is key to conductivity. Increasing the number of aromatic amino acids increases conductivity, whereas pilins with lower aromatic amino acid abundance or large aromatic-free gaps in the pilin sequence yield wires with lower conductivities [18,22,25,28–33]. Close packing

of aromatic amino acids during filament assembly of pilin-based wires, forming paths for aromatic-to-aromatic electron conduction, is the likely mechanism for electron transport along the length. As previously reviewed in detail [16,34] there is debate over the fine-scale details of whether electrons move via electron hopping or a metallic-like conduction. However, these specifics do not impact the practical benefit that the conductivity of pilin-based wires can readily be tuned over 1 million–fold by simply designing pilin genes with different abundances of aromatic amino acids [23].

Other functional aspects of pilin nanowires can also be modified with the design of synthetic pilin genes. For example, encoding additional amino acids at the carboxyl end of pilins yields wires in which the added peptides are displayed on the outer surface [10]. As detailed in a subsequent section, addition of peptide ligands in this manner may aid in the design of pilin nanowires for sensing functions (Figure 2). Modifying the properties of nanowires is also likely to be a useful approach for adhering wires to specific surfaces, enhancing interactions with polymers to produce flexible electronics, and increasing biocompatibility [10]. The stoichiometry of peptides displayed on wires can be controlled by expressing multiple pilin genes, each under the control of inducible genetic circuits. This approach has also been used to express wires with multiple peptide ligands displayed on the same wire [10].

Commercial scale production of pilin-based wires seems possible (see the section 'In Vivo versus In Vitro Fabrication of Protein Nanowires'). Conductive pilin-based wires can be expressed in E. coli grown aerobically and harvested with simple filtration [11]. Growing E. coli aerobically is easier and faster than growing G. sulfurreducens anaerobically and offers the additional advantage that, unlike G. sulfurreducens, E. coli does not express outer-surface cytochromes, avoiding the potential contamination of pilin-based wires with cytochromes [31,35]. The extensive genetic
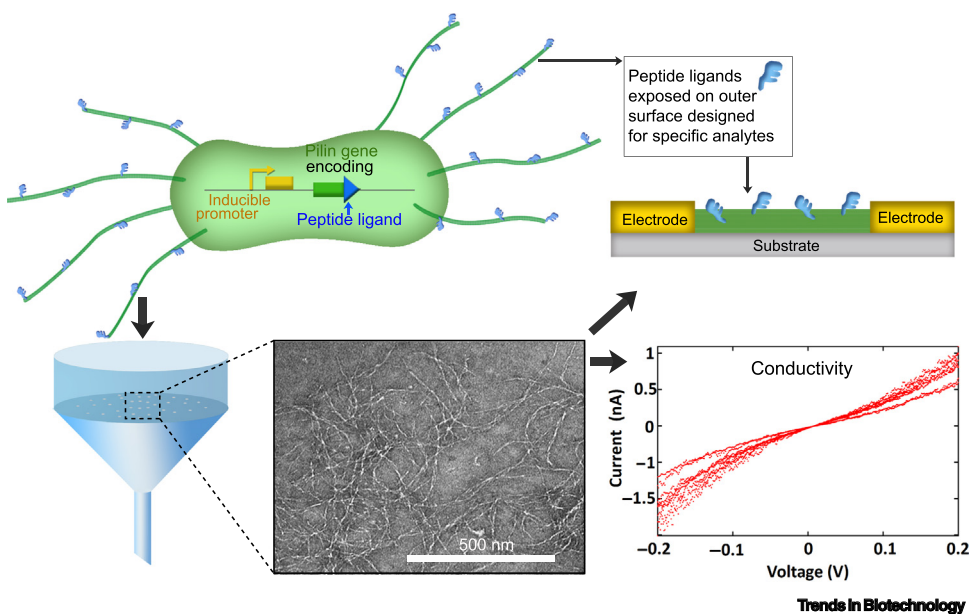

Figure 2. Strategy for Producing Pilin-Based Nanowires with Peptide Ligands That Can Confer Specific Sensing Functions or (Not Shown) Aid in Binding Wires to Surfaces or Linking to Polymers for Composite Formation. An inducible system controls the expression of a synthetic pilin gene which encodes a peptide ligand that is expressed on the outer surface of the pili. Pili sheared from the cells (protein nanowires) are collected with filtration. Individual wires are conductive, and thin films of wires can function as sensor components.

toolbox available for *E. coli* opens broad possibilities for protein nanowire design, including the potential to design wires that incorporate unnatural amino acids.

As detailed below, nanowires composed of native pilins from *G. sulfurreducens* have yielded promising results in multiple types of electronic applications. The native wires are highly robust, functioning well in these applications for periods of many months without failure [14,36]. Pilin-based wires tolerate high temperatures (105°C, the highest tested [37]) and exposure to solvents (Y.-L. Sun, *et al.*, unpublished). These are important considerations for many types of electronics fabrication. Pilin-based wires can be assembled into 'tapes and cables' in which the individual wires are highly aligned (Y.-L. Sun, *et al.*, unpublished) and also can be incorporated into polymers to produce flexible conductive composite materials [37].

Curli Fibers Modified with Aromatic Amino Acids

Curli fibers are composed of the monomer protein CsgA, which is secreted from *E. coli* and self-assembles extracellularly into filaments that, in the native state, are poorly conductive [38]. Expression of curli fibers modified with metal-binding peptides displayed on the outer surface of the fibers is a strategy for metallization of curli fibers, increasing conductivity [39,40]. However, the addition of metals introduces a component that is expensive and energy intensive to produce and that is also nonbiodegradable.

Expression of synthetic genes designed to yield modified CsgAs with closely positioned aromatic amino acids (aromatic rings within a few angstroms) yielded curli fibers that could be harvested as films that were more conductive than films composed of wild-type curli fibers [41]. Higher conductivities were associated with higher aromatic amino acid content. The conductivity of individual nanowires was not investigated in these studies, precluding direct comparisons with the conductivity of pilin-based nanowires. In an alternative approach, aromatic amino acids were added to the carboxyl end of CsgA, with the goal that aromatic amino acids in bundled curli fibers would form a conductive network [42]. This resulted in *E. coli* biofilms with higher conductivities than the wild-type strain, but the conductivity of fibers separated from cells was not evaluated.

Further investigation into the potential for introducing intrinsic conductivity into curli fibers for producing sustainable electronic materials is clearly a promising approach for additional development of intrinsically conductive protein nanowires. Prospects for scale-up of curli fiber production are very promising because curli fibers can be produced with aerobically grown *E. coli* and harvested with simple, scalable filtration methods [43].

Cytochrome Wires

Under some conditions, the microbe *Shewanella oneidensis*, which is typically rod shaped, forms protrusions of the outer membrane and periplasm that are micrometers in length and rich in multiheme *c*-type cytochromes [44]. The membrane extrusions can be harvested by shearing [45]. When dried and chemically fixed, the membrane extensions shrink to form 10-nm-diameter filaments with a conductivity of ~1 S/cm [46]. Although it is has been proposed that these filaments might serve as the conductive component in electronic devices [45,47], the stability of this complex composite of cytochromes, other proteins, and lipids is unknown. Conductivity has only been documented after fixation with glutaraldehyde, a toxic reagent not suitable for sustainable large-scale production. Production is also limited by the fact that cells rarely produce more than one filament per cell [44], providing a low filament/cell yield.

Under some growth conditions, 4-nm-diameter wires composed solely of the six-heme *c*-type cytochrome OmcS [35,48] or 2.5-nm-diameter wires composed of the eight-heme *c*-type

cytochrome OmcZ [49] can be recovered from outer-surface protein preparations of *G. sulfurreducens*. The conductivity of individual OmcS wires (~30 mS/cm) [49] and OmcZ wires (~12 S/cm at pH 7) [49] could be suitable for the development of some types of electronic devices. Applications for **cytochrome wires** have not yet been described, and it has been suggested that cytochrome wire mimetics may be more suitable for applications because cytochrome wires could be 'difficult to engineer and repurpose' [50].

Producing cytochrome wires with *G. sulfurreducens* faces not only the challenges of large-scale cultivation of a slow growing anaerobe but also the fact that the abundance of cytochrome wires appears to be highly variable, depending on the strain of *G. sulfurreducens* and growth conditions. In some instances, cytochrome wires predominate [48]; in others, there is a mixture of OmcS wires and pilin-based wires [35]; and in other preparations, all the wires had diameters consistent with pilin-based wires [13]. All of the filaments emanating from cells appeared to be pili in a strain in which the pilin monomer was modified with a peptide tag that enabled filament identification with immunogold labeling [10]. With another strain, immunogold labeling for OmcS revealed, depending on the growth phase, no OmcS filaments or a mixture of labeled and unlabeled filaments [51]. OmcS may also associate with pilin-based filaments [23,51–53]. Heterologous expression of cytochrome wires in common laboratory production strains, such as *E. coli*, may be challenging due to a lack of known systems for exporting cytochromes to the outer surface. However, strains of *G. sulfurreducens* that overexpress OmcS [54] or OmcZ [55] have been constructed, which may facilitate expression of cytochrome wires on the outer cell surface and/or the production of monomers that can be assembled into wires *in vitro*.

Protein Nanowires from Cable Bacteria

Electrically conductive filaments can be extracted from cable bacteria by chemically dissolving other cell components [56]. Cable bacteria live in aquatic sediments, forming chains of thousands of connected cells that transport electrons derived from sulfide oxidation in submerged sediments to oxygen in the overlying water [57–59]. The conductive filaments extracted from the cells are an array of about 60 parallel fibers (individual fiber diameter ~50 nm) [56,60]. Conductivities of individual fibers are typically 1–20 S/cm [56]. The conductive filaments are not composed of cytochromes [56]. The strongest candidate for the primary structural protein is an aromatic-rich pilin that may form conductive protein structures held within a carbohydrate matrix [61].

Further elucidation of the structure and function of the cable bacteria conductive fibers is required in order to evaluate their potential application in electronic devices. As currently extracted from cells, conductivity is lost upon exposure to air [56], a major drawback for many applications. Cable bacteria have not been recovered in pure culture and are extremely difficult to grow, limiting the possibilities for mass production or genetically tuning conductivity or filament functionalities. Heterologous expression of cable bacteria filaments in more readily cultured microbes is not currently a feasible route, because the capacity for producing intracellular filaments with a morphology like that of cable bacteria filaments has been found only in cable bacteria.

In Vivo versus *In Vitro* Fabrication of Protein Nanowires

A wide diversity of peptides that self-assemble *in vitro* into conductive filaments have been described [4,34,38,62,63]. A detailed discussion of these materials is outside the scope of this review. However, it is important to note that *in vitro* assembly of protein nanowires is a strong contender with microbial production as a route for commercial scale production of protein-based electronics [34,62–64]. A potential advantage of *in vitro* fabrication is that it could avoid potential concerns about impurities associated with *in vivo* fabrication and purification that might impact electronic device performance or, in the case of biomedical applications, trigger an immune response [62].

Truncated *G. sulfurreducens* pilin monomers were assembled into conductive filaments *in vitro*, but this method required an assembly buffer of toxic solvents, as well as solvent evaporation and multiple other technically complex steps that would prevent sustainable, scalable protein nanowire production [65]. Initial studies with water-based assembly of biomimetic peptides have shown more promising results [64,66–68]. Notably, low concentrations (100–200 μM) of a synthetic peptide designed to yield a tightly packed aromatic core assembled into nanowires (2.3-nm diameter) with a conductivity of 1 S/cm [68]. However, the nanowires had a high propensity for aggregation and *in vitro* assembly at millimolar concentrations of peptide yielded gels with declining gel conductivities as peptide concentrations were increased [68]. Conductivity was dependent upon maintaining a fiber morphology, which was only possible at low peptide concentrations. These are not favorable conditions for scaled-up fabrication. By contrast, cells maintain the proper environment during *in vivo* fabrication of pilin-based nanowires, assembling wires with a sophisticated cellular machinery [69] that consistently yields a highly reproducible and uniform product.

Difficulties were also reported in trying to design nanofilaments to replicate long-range cytochrome-based electron transport. Thin films of cytochromes aligned along the filaments did not have a statistically significant higher conductance than thin films of cytochromes not on filaments [50].

As applications for protein nanowire-based electronics begin to be developed, the requirements for cost-effective, commercial scale production become an important consideration. A diversity of commodities are made with *E. coli* from inexpensive, renewable feedstocks [70]. This approach should also be applicable to the commercial scale fabrication of pilin-based nanowires [11] or conductive curli fibers [41]. Processes based on *in vitro* assembly of synthetic peptides may be difficult to scale up [71]. The cost of producing and purifying peptides is high, and the careful maintenance of conditions to ensure nanowire production at high yield may be technically challenging at large scale. The environmental sustainability of peptide synthesis, which may require toxic reagents for synthesis and purification, must also be considered. Thus, as better strategies for *in vitro* fabrication of electrically conductive protein nanowires are developed, technoeconomic analytical comparisons of *in vitro* and *in vivo* fabrication will become highly relevant.

Fabricating Novel Devices with Protein Nanowires

The potential to mass produce protein nanowires with *E. coli*, as well as the future possibility of *in vitro* fabrication, make commercial scale production of electronic devices with protein nanowires conceivable. Several types of proof-of-concept electronic devices have already been demonstrated.

Sensors

Sensors are a frequently envisioned application for nanowires [72,73]. Several types of sensors (Figure 3) have been fabricated with thin films of *G. sulfurreducens* nanowires harvested from cells grown under conditions that appeared to yield predominately pilin-based wires [13]. One device was highly responsive to ammonia gas over a range of ammonia concentrations [10 parts per billion (ppb) to 1000 parts per million (ppm)] relevant for industrial, environmental, and biomedical applications [14]. Other common gases did not interfere with the analysis. Flexible, wearable devices that could continuously monitor skin hydration or breathing rate or that could function as a touchless sensor that could track finger location have also been developed [36].

A broader range of sensors may be developed with nanowire functionalization. The surface of silicon nanowires and carbon nanotubes are functionalized with aptamers or antibodies to achieve specificity and sensitivity in analyte detection [72,73]. Analyte binding influences nanowire/
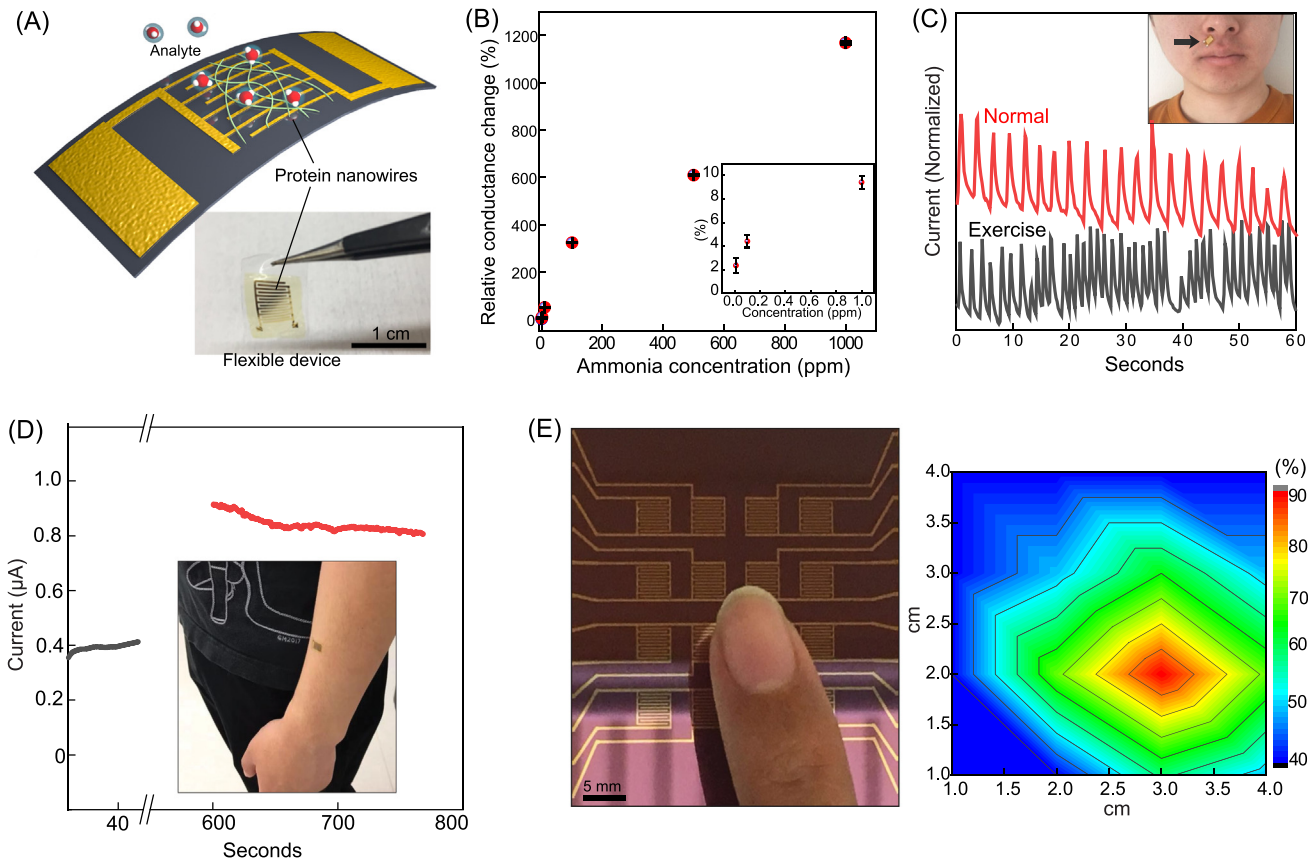
Figure 3. Proof-of-Concept Sensor Devices Produced with Protein Nanowires from *Geobacter*. (A) Schematic (top) and photo (bottom) of sensor design. (B) Ammonia gas sensing, demonstrating high sensitivity and conductance change over a wide range of ammonia concentrations. Inset provides higher resolution for the response from 10 parts per billion (ppb) to 1 part per million (ppm). (C) Wearable humidity sensor placed close to the nose to monitor respiration rates at normal resting state and after exercise. (D) Sensor detects different humidity levels on the arm before (gray) and after (red) exercise. (E) Noncontact detector 4 × 4 sensor array (left), which tracks the position of a fingertip placed above the sensor matrix (right). Adapted, with permission, from [14] (B) and [36] (C–E).

nanotube conductivity. However, the size (2–10 nm) of the functionalization groups can introduce a 'spacer' effect that separates the analyte from the nanowire surface, weakening the sensor response. As noted above, short peptides that function as ligands are readily introduced on the outer surface of pilin nanowires (Figure 2), and peptides for specific binding of a broad range of analytes of environmental and biomedical importance are already known [10]. The close binding of analytes to the pilin nanowire surface is expected to result in a strong sensor response [10]. Tailoring pilin nanowires for specific sensor function will likely be an area of intensive future research.

Sustainable Electricity Production

Preliminary studies on humidity sensing [36] led to the unexpected finding that thin films (2–18 μm) of *G. sulfurreducens* protein nanowires can produce electricity in the ambient environment [12] when they are connected to electrodes in the proper configuration (Figure 4). Exposure of the upper layer of the nanowire film to atmospheric humidity leads to a vertical moisture gradient within the film that is associated with a constant output voltage of ~0.5 V [12]. A likely explanation for this voltage is that an ionization gradient develops due to differences in the degree of proton disassociation at different moisture contents within the film. At steady state, water adsorption at
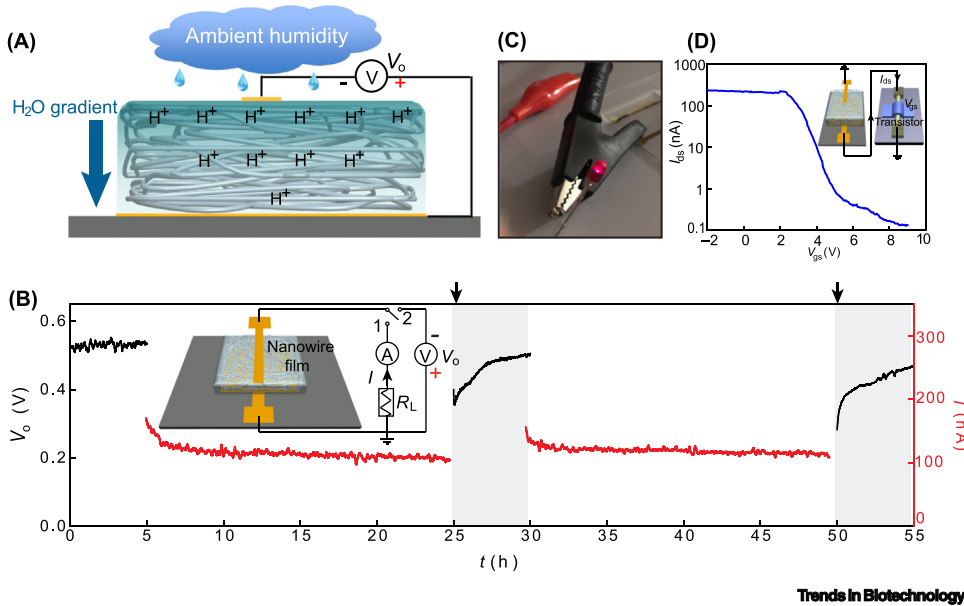
Figure 4. Harvesting Electricity from Ambient Humidity with a Thin Film of *Geobacter* Wires. (A) Water adsorption from atmospheric humidity at the upper surface results in water and proton gradients. (B) Sequential periods of continuous current production for 20 h. The slight voltage drop after current consumption is reestablished during self-recharge (gray). (C) Charging a capacitor provides power to light a light-emitting diode (LED). (D) Sufficient current is produced to power a transistor. Abbreviations: I, output current; Ids, transistor drain-source current; RL, load resistor; Vgs, transistor gate voltage; Vo, open-circuit voltage. Adapted, with permission, from [12].

the thin-film surface is matched by evaporation, and thus a likely source of the energy for long-term current production is energy release associated with adsorption [12].

Current could be harvested for over 1 day without a significant decline in output and only small drops in voltage (Figure 4). The voltage could be restored with only several hours of recharge without current draw. The films were durable, continuing to produce the same amount of current after 10 months of device exposure to the ambient environment [12]. Devices of 1 mm^2 yielded current outputs as high as 400 nA (nanoampere) [12], and the current output of thin films was sufficient to power a transistor [12] or a wearable electronic sensing device [36]. Voltage or current scaled linearly when devices were connected in series or parallel, and multiple connected devices could charge a capacitor to power small electronic devices [12]. The potential for larger scaling output through vertical stacking of multiple devices separated by air gaps could lead to volumetric outputs to rival photovoltaics while avoiding the need for light and its associated intermittency. Furthermore, current output was only a fraction of the theoretical limit, suggesting that manipulation of nanowire structure through the design of new synthetic pilins might increase output. Although various **hydrovoltaic technologies** are also being developed with non-biological carbon nanomaterials [74], none yet match the current-generating capacity of microbial nanowire thin films in the ambient environment.

Neuromorphic Devices
The potential of pilin-based nanowires to transfer electrons to metals [23] was the basis of a proof-of-concept **memristor** device designed to function as an analog of biological neurons and synapses [13]. Memristors mimic the history-dependent recording of inputs by the brain. Increasingly better electrical connections are established between two electrodes with increased duration or frequency of an electrical input signal [75,76]. To produce the microbial nanowire-based memristor, a thin film of *G. sulfurreducens* wires was sandwiched between two silver

metal [Ag(0)] electrodes. Silver ions (Ag$^+$) entered solution from the abiological, electrochemical oxidation of Ag(0) at the anode. The electrons donated by Ag(0) at the anode were transferred to the cathode electrode and were available for reduction of Ag$^+$ to Ag(0). The nanowires appeared to function as a catalyst to lower the overpotential for Ag$^+$ reduction in the space between the two electrodes. The Ag(0) formed enhanced the electrical connection between the electrodes. This was achieved at potentials (40–100 mV) comparable with the voltages at which biological neurons function and enabled memory of low-voltage electronic outputs mimicking biological signaling [13]. These results demonstrated that artificial neurons and synapses can be constructed to strictly match the signal processing in biological systems. Previously described memristors operate at higher voltages (0.5–2 V) [77,78] and thus are not well matched for interfacing with biological systems. The results with the microbial nanowire-based memristor may help guide the design of new materials with broad potential in ultralow-power computing.

Concluding Remarks

The fabrication of electronic devices with microbially produced protein nanowires is clearly in its infancy (see Outstanding Questions). However, the available demonstrations of novel device function and robust nanowire properties, coupled with the availability of an *E. coli* chassis for large-scale nanowire production, suggest that pilin-based wires have significant potential as a sustainable electronic material. In addition to their sustainability and unique properties for sensing and electricity generation, pilin-based wires, or self-assembling peptide-based mimetics, may be particularly well suited for making electrical connections with cells for biomedical applications because of the well-known capacity for pili to bind to eukaryotic cells and the possibility that they can be designed to degrade in the human body when no longer required [62]. High biocompatibility is expected but has not yet been verified. With further investigation, nanowires assembled from other microbial products, such as curli fibers, cytochrome wires, or the component(s) that form the conductive fibers of cable bacteria, may also prove to be attractive choices for electronic applications. For each new material and application, cost–benefit assessments versus more traditional nanowire materials will be required.

Realization of the full potential of microbial protein nanowires will require substantial further basic research. For example, studies to date have primarily focused on the conductivity estimates that were initially required to evaluate biological function or, in the case of curli fibers, to establish that aromatic amino acids that were introduced conferred conductivity. Needed now is fundamental research on nanowire structure and the mechanisms of charge transport, as well as an evaluation of the optical, thermal, and mechanical properties, to serve as a foundational knowledge base to support development of new applications. For example, for most electrically conductive protein nanowires, even the most basic information on electronic characteristics such as mobility, band structure, charge density, and capacitance has not been definitively determined. Interdisciplinary research teams with expertise in biophysics, biochemistry, electronics, and microbiology will be a must for rapid progress.

Outstanding Questions

What additional types of intrinsically conductive nanowires might microbes produce, and how can these and recently discovered microbial nanowires, such as cytochrome wires and cable bacteria fibers, be adapted for electronic applications?

Can the production of protein nanowires and electronic fabrication methods be scaled to commercially viable levels?

What is the scope of new electronic properties and sensing applications that can be developed by further modifying microbial nanowire structure?

How can microbial nanowires be adapted for biocompatible wiring to eukaryotic cells?

Can ambient energy harvesting with protein nanowire devices be increased to provide power beyond generation for small-scale electronics?

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