nature reviews bioengineering

https://doi.org/10.1038/s44222-024-00262-6

Review article

Check for updates

Integrating bioelectronics with cell-based synthetic biology

Jonathan Rivnay[1,2,3] ✉, Ritu Raman[4], Jacob T. Robinson[5,6,7], Christian Schreib[6,7], Tzahi Cohen-Karni[8,9], Kate E. Galloway[10] & Omid Veiseh[6,8,11]

Abstract

Biohybrid devices based on engineered cells interfaced with bioelectronics represent a promising union where the strengths of each field can be synergistically combined, resulting in constructs with properties that are not otherwise achievable. Recent progress in biomaterials and cell-based synthetic biology has resulted in cells that can be remotely triggered via multiple modalities and can access a number of cellular pathways to achieve complex sensing and biomolecule production tasks. Although these living cells can be deployed as next-generation diagnostics and cell-based therapies, they are limited by the fundamental boundaries of biology. Bioelectronics, conversely, has been engineered to leverage the strengths of established computational hardware and software, integrates multiple inputs of biometric and external data, and allows communication over long distances. However, bioelectronics often requires considerable power to perform complex tasks and lacks the specificity and adaptability of cells and tissues. The parallel advances in synthetic biology, biomaterials and bioelectronics therefore present new opportunities in devices for regulated cell therapies, diagnostic tools and next-generation robotics. In this Review, we discuss the enabling mechanisms of communication between engineered cells and bioelectronics platforms, describe the approaches and challenges in assembling and deploying such systems, and highlight recent prototypes. The continued advancement in cell support systems and both internal and external closed-loop control suggest forthcoming breakthrough opportunities for biohybrid bioelectronics.

Sections

A full list of affiliations appears at the end of the paper. ✉e-mail: jrivnay@northwestern.edu

Key points

- Biohybrid devices bring forth new opportunities not achievable by bioelectronics or synthetic biology alone, enabling function that overcomes fundamental biological processes while maintaining connections to the Internet of Things and stakeholders.
- Biohybrid devices can enable real-time data-driven patient care while improving adherence and enhancing patient access by enabling remote treatment and monitoring.
- Combining cell engineering and bioelectronics requires efficient means of bidirectional communication between cells and abiotic bioelectronics, which can be achieved across modalities.
- Many challenges exist, including prevention of fibrosis or fouling for implants and life support to sustain cells, which can be addressed via co-design of biomaterials, bioelectronics and/or cell engineering.
- While key demonstrations have been achieved for therapy, diagnostics and robotics, the examples are largely at the proof-of-concept stage.
- Future advances in longevity, feedback and regulation, and multicellular approaches are needed to advance the field.

Introduction

Biohybrid electronics brings together two research communities that have evolved largely independently but are keenly aware of, and opportunistically leverage, key tools from one another. Specifically, recent advances in both bioelectronics and synthetic biology have created complementary toolboxes that, when combined, enable devices with capabilities surpassing the use of either field's tools alone[1] (Fig. 1 and Table 1). For example, where bioelectronics show poor selectivity for stimulation, cellular engineering strategies developed over decades can impart cell-type level specificity. Where biological systems suffer from slow signal transmission over millimetre-scale distances, bioelectronics can wirelessly communicate to loop in the user or operator, as well as local and global (Internet of Things) environmental signals and data. It is thus not surprising that the union of these disciplines, called living electronics, biohybrid (bio)electronics or {syn(bio}electronics), brings great potential across multiple domains: health monitoring and therapy, responsive environmental tools and robotics, to name a few. To best understand this union, it is critical to understand the strengths (and limitations) of bioelectronics and synthetic biology independently.

Engineered living (multi)cellular organisms can be viewed as living machines that have, over millennia, evolved energy efficient, highly parallelized and specific functions. These functions, inherent to the sub-cellular to tissue-scale living constructs, can be harnessed for biomanufacturing, sensing and logic, and microscale force transduction and, importantly, can communicate via myriad cues (chemical, mechanical, optical, electrical). Engineered cells can be used for on-site production of biomolecules: so-called cell therapies, which target living, engineered cells to be implanted or injected as a therapy[2,3]. These cells use their own machinery to synthesize peptides and proteins, which a host is unable to produce sufficiently, to treat diseases such as cancer, diabetes and other endocrine disorders. Similarly, living mammalian cells and bacteria can sense their environment using native receptors and signalling cascades or via engineered pathways integrating non-native cues[4–6]. Beyond simply carrying (often on the plasma membrane) a specific receptor to bind or detect a molecule or physical input, multiple functions can be integrated within the same cell. Different cells can be multiplexed, logic circuits can be implemented, and signals amplified and even stored as on-board memory, before being communicated to the user through an output signal (that is, electrical or fluorescence signals). In this way, for example, compact and parallel diagnostics can be performed to monitor waterways or to track inflammation in vivo or glucose availability in the gastrointestinal tract[4,6,7]. Furthermore, specific cell types and their tissues can be used to harness their specific evolved function. For example, muscle cells can be harnessed for their efficient force production in microscale regimes[8], a feature that is otherwise challenging to achieve with synthetic soft actuators. Biological systems are capable of self-contained regulated control at cellular, tissue and organism levels by combining the sensing and logic functions with actuation (biosynthesis and force transduction), which is at the core of the autonomy of living matter.

Living tissues can leverage genetic editing tools to programme multiple features that extend their function beyond what is found in nature: synthesis of non-native products, enhanced productivity, improved resilience to senescence or cell death, tailored non-native stimuli response and programmable safety switches (Box 1). Critically, these engineered living systems are, by their very nature, at the mercy of biology. Their response times are limited by biological mechanisms such as molecular rearrangements, diffusion, and transcription and translation. Many early cell engineering strategies were established in prokaryotes, such that they can respond to and sense a variety of external stimuli, setting the stage for similar innovations in mammalian cells. In engineering cells, especially mammalian cells, it can be challenging and inefficient to incorporate multiple gene circuits within a single cell[9–12]. Likely the largest barrier to implementation is that cells must be kept alive and functional to serve their engineered function and they require energy, ideal environmental conditions (pH, temperature) and waste removal.

Bioelectronics describes the interface of biological systems and electronics, whereby electronic components as well as their interconnects and integration platforms are entirely abiotic, relying on inorganic materials (metals, ceramics), with polymers often relegated to passive, insulating layers. The use of polymer-based or carbon-based electronic materials has gained interest, especially at the biotic–abiotic interface, and these are often called 'organic' bioelectronics (describing the use of materials made up of low-mass elements common in biology), even though they do not use living materials. Bioelectronics thus considers the electronics and encapsulation materials as the engineered system, with living or environmental systems (biology) being external to a device. In this sense, a biological system is the device's environment, an entity to be sensed and acted on. The interfacial nature and form factor of a device largely dictate the efficiency and stability of bidirectional information transfer. Bioelectronics leverages building blocks from electronics: complementary metal oxide–semiconductor (CMOS) technology enables pre-defined tasks to be hardwired into a device or to be programmed externally. Logic operations can be rapidly executed at time scales that are orders of magnitude faster than biology. Finally, information can be passed from and integrated with other electronic systems — connecting to local networks of sensors and actuators, to cloud services for later analysis, or remotely controlled by

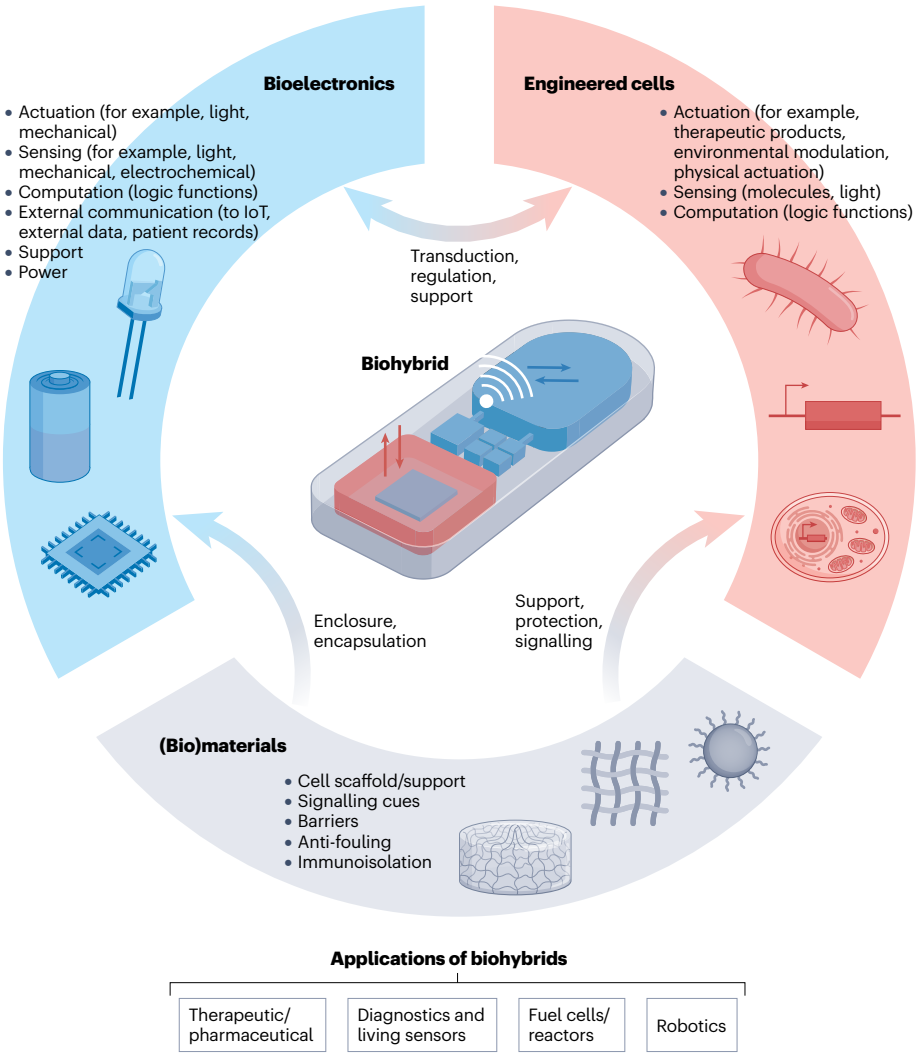


Fig. 1 | Biohybrid bioelectronic components, function and applications. Bioelectronic components (blue), engineered cells (red), and functional encapsulation and biomaterials (grey) serve as building blocks in biohybrid systems. Their assembly and interactions form various co-dependencies which the different subsystems depend on for functional operation or longevity. IoT, Internet of Things.

other people or electronic systems. In this way, a user or patient, a health practitioner, or a family member can receive actionable data as well as control or supervise an operation or therapy. Bioelectronics thus promises boundless potential, for example, in providing precision dosing and timing, ranging from pacemakers to deep brain stimulators emitting electrical pulses of precise frequency and intensity to control muscle and neural tissues or fluidic pumps controlling dosing of therapies. Bioelectronic systems can leverage sensing data to regulate actuation. The classical example is an insulin pump, which senses glucose levels and delivers insulin at the appropriate levels; however, more complex multi-sensor e-skins are in development that will enable prosthetics or robots to respond to their environment more naturally[13,14]. Bioelectronics can perform these functions in both a closed-loop and human-in-the-loop manner. With a rise in neuromorphic computing and machine learning, new approaches to processing data, alongside existing information, enable electronic systems to classify objects, scenarios and diagnoses, with the potential to adapt and learn over time.

However, bioelectronic systems are incompatible with wet, salt-rich environments, and often have stringent requirements for large power and small form factors. Electrochemical processes, especially unintended ones owing to aqueous exposure to electrical currents or voltages, can damage electronic components, cause short circuits, or expose the biological environment to harmful chemical or physical stimuli. Communication through tissue and over long distances, although largely more effective than biological communication, can be power intensive. The Achilles' heel of bioelectronics is the need to provide electrical power, which is not readily available in usable forms (by engineered electronics) in biological settings, thus necessitating batteries or bulky transceivers. Much attention has been given to these challenges, which will result in new materials and coatings for stable barriers[15], hardware advances to handle intermittent power, long-term storage and power harvesting, and wireless power delivery through the body[16]. As with any technology with wireless connectivity, safety and information security must be considered and properly engineered.

Unlike traditional bioelectronics, in a biohybrid device, the boundary of the engineered system should encompass both bioelectronic and living cellular components, which interact with other living or environmental systems. To engineer biohybrid bioelectronics, especially

Table 1 | I Comparison of strengths of cell engineering (synthetic biology) and bioelectronics

Feature	Synthetic biology		Bioelectronics	
Long-range communication	■	Signal velocity typically <1 μm s^{-1}. Largely limited to direct, physical, chemical communication[a]	■■■	Signal velocity typically near 3×10^8 m s^{-1}. Multimodal and wireless communication
Latency	■	Typically 0.01-100 s	■■■	Typically less than 0.1 s
Ease of programming	■	Lack of standard programming languages	■■■	Many widely used standardized programming languages
Physical actuation	■■	Muscle generates force efficiently in microscale and macroscale regimes with higher mass to power ratio than abiotic actuators. Electrical and optical actuation possible	■■■	Fast, precise actuation of light, magnetic and/or electric field, and heat with standard optoelectronic components. Motors, piezo and pneumatic actuators produce mechanical forces in the macroscale regime
Physical sensing	■■	Receptors for mechanical force, light, electric fields and heat	■■■	Fast and precise sensors for mechanical force, light, electric and magnetic fields, and heat
Feedback control	■■	Limited precision and complexity of feedback control	■■■	Many complex and accurate feedback controls systems
Security	■■■	Inherently secure in operation[b]	■■■	Robust encryption and cyber security
Safety in failure	■■	Can cause disease or injury[c]	■■	Can cause injury[c]
Chemical actuation	■■■	Capable of producing many biomolecules	■	Limited mainly to redox reactions, or pre-loaded reservoirs of few or limited drugs
Chemical sensing	■■■	Receptors for most biomolecules	■	Limited mainly to molecules with redox signatures, or integration with known biorecognition elements. Limited long-term stability
Self-renewal	■■■	Most cells can self-replicate		Most electronics cannot self-replicate

Number of squares (■) denotes qualitative strength of a given feature, with examples or relevant metrics denoted for each. [a]Bioluminescence can provide long-range signalling but light intensity is typically too low to activate biological activity[48]. [b]For autologous cells, patient genetic information can be compromised during cell production but robust electronic records and encryption can be implemented. [c]Both engineered cells and bioelectronics can incorporate emergency and/or automated shut offs — cells can be engineered with pharmacological 'safety switches'.

with living cells and tissues, system design and integration require transdisciplinary approaches and co-design strategies. In this Review, we introduce and outline the toolbox that enables biohybrid bioelectronics. We then outline the modes and mechanisms with which cells and bioelectronic systems communicate (for both sensing and actuation or stimulation), including electrical, electrochemical, optical and mechanical mechanisms. Cell engineering strategies enabling engineered cell function for biohybrids will be highlighted. Subsequently, we describe the challenges and efforts to achieve integration between bioelectronic and cellular components. Such systems require not only the sensing and actuation of each component but should efficiently transduce signals between the bioelectronic and living cellular systems. The same tools that enable robust coatings and matrices to protect electronics and cells from degradation and isolation are as critical in a hybrid device, yet bioelectronics and engineered cells themselves can serve an additional role as a life-support system to maintain and report on the health and viability of the cells and tissues. Finally, we highlight key examples of demonstrated bioelectronic biohybrid prototypes that showcase the complementarity of biohybrid devices, spanning from robotics to diagnostics and therapeutics, and provide an outlook for biohybrid devices identifying future opportunities.

Modalities of cell–bioelectronic communication

To link engineered cells and electronics an interface must be built to exchange information between living cells and non-living electronic components. This 'bidirectional' communication must include both 'writing' (that is, actuating biological activity in engineered cells and tissues) and 'reading' (that is, recording signals produced by the living tissue). These biohybrid building blocks form the toolbox that is necessary to build biohybrid electronic devices or systems (Fig. 2). The primary challenge in these biohybrid read–write systems is that information in electronic circuits is transmitted by electrons and holes, whereas information in living cells is typically transmitted by ions and molecules. Thus, technologies for reading and writing must convert between these different types of information carriers. Below, we describe the principal methods for converting between biotic and abiotic information carriers, which provide the basic transduction technologies required for biohybrid bioelectronics.

Electrical reading and writing

Since the 1700s scientists have recognized that electrical currents can activate nerve and muscle activity and that this activity can also be recorded by measuring the electrical currents or potentials produced by living tissue[17]. The principle of this electronic transduction is that ions flowing across the cell membrane through ion channels create an ionic current in the electrolytes surrounding the tissue. Thus, electrical readout technology primarily records the activity of neurons or muscle cells because activity in these cells is associated with current flowing through voltage-gated ion channels in the cell membrane. Fortunately, recent advances in genetic engineering have enabled researchers to engineer clonal cell lines, such as human embryonic kidney (HEK) cells, to generate action potentials by expressing voltage-gated sodium and potassium channels[18,19]. Thus, a biohybrid electronic readout can be engineered by using these 'spiking HEK' cells along with electrodes or transistors to record cell activity. By engineering these cells to generate an action potential in response to physiological signals, it would be possible to create an electronic biohybrid readout system.

For electrical stimulation or writing, electrons can be moved to or from the electrode surface to drive ions through the electrolyte. This ionic current can, in turn, stimulate voltage-gated ion channels and drive cellular signalling. For example, an electrode can be used to induce voltage-gated calcium currents that drive engineered gene

circuits in a process referred to as 'electrogenetics'[20–23]. In either case, the electrodes used to drive cell signalling benefit from electrode materials that have large charge injection capacity, which describes the amount of charge that flows from the surface of the electrode per unit area at a given potential. The electrode characteristics and their efficacy in both recording and stimulation are largely governed by their size and/or shape as well as interfacial impedance, which can be tailored through the properties and morphology (that is, roughness, porosity) of the materials[24].

(Electro)chemical reading and writing

Chemical reading and writing typically provide better chemical and cellular specificity when compared to direct electrical methods but are often hindered by longer stimulation and recording latencies. Traditional chemical stimulation and measurement through intravenous delivery, drug pumps and controlled delivery devices, or blood draws are the most common but also have the longest latencies because drugs must diffuse through the blood or tissue. Faster chemical reading and writing can be achieved by creating or sensing chemicals near the target using electrochemical reactions. Rather than moving ions through the electrolyte with an electrode, electrochemical methods can stimulate or record cell activity by creating or sensing ions or electroactive species directly. These methods require tight control over the electrode surface and electrical potential to avoid undesirable chemical reactions. For example, dopamine, a key neurotransmitter, can be measured using fast-scanning cyclic voltammetry and carbon-based electrodes[25,26]. For sensing non-electroactive molecules, biochemical detection based on biological or bio-inspired recognition elements (such as enzymes, antibodies or aptamers) associated with electrodes or transistor interfaces benefits from decades of development[27,28]. In this case, changes in charge or current associated with biochemical reactions at the surface of functionalized electrodes enable selective sensing; however, continuous and resilient long-term sensing in complex environments remains a challenge.

Redox-active molecules are the basis for electron transfer in biology; therefore, reducing or oxidizing chemical species at the electrode

Box 1 | Synthetic biology tools for engineering cells for biohybrid devices

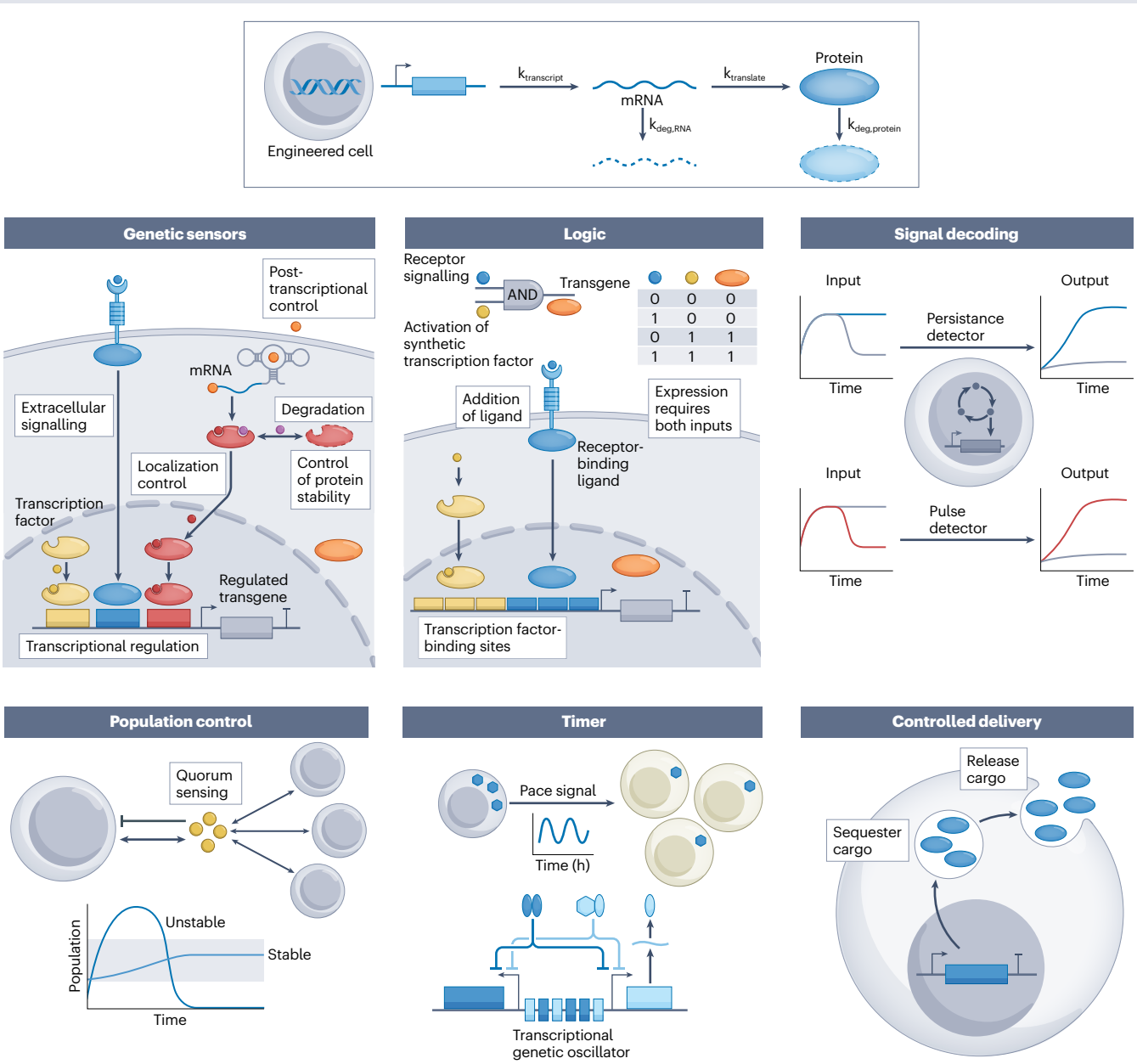
Biohybrid systems harness the exquisite sensing and programmability of cells via native and augmented networks of biomolecules. Increasingly, biohybrid systems integrate synthetic biology tools to perform sophisticated computation and enact precise control of cellular responses[163–166]. Building cells that can respond to the desired cues requires the construction of interfaces to receive and relay information[150,151,167].

Transgenic systems and genetic elements are encoded through DNA sequences that are integrated into the host genome for stable expression. Modular sets of genetic sequences can be called genetic elements or 'parts'[168–170]. These parts can encode the rate at which genes are expressed by varying rates across the central dogma (Box Fig. 1), including synthesis, degradation, and transport of RNAs and proteins. The types of elements required for gene expression vary by species as the process of gene regulation varies widely between bacteria, yeast and mammals. For all species, coding genes specify the sequences that will be expressed as proteins. By recruiting RNA polymerases, the promoter controls the rate of transcription. For eukaryotes, the exact start site and end site of transcription can vary by promoter and by cell type, thus encoding different untranslated regions. These untranslated regions can influence mRNA processing, stability and translation, shaping the pattern of gene expression and enabling cell-type-specific profiles and responses.

Connecting sensors to regulate the transcription, translation and release of biomolecules closes the control loop, enabling both user-guided and autonomous control. Although sensor molecules can combine actuation, transmission of signals into synthetic gene networks can provide integration of multiple signals and sophisticated processing[171–173]. Bacteria, in particular, have been genetically modified to exhibit precise responses to electrical stimuli such as light, electric fields and magnetic fields, allowing for the control of gene expression and cellular behaviour with high spatial and temporal resolution[174–176]. They have also been used to sense a variety of different signals such as disease markers[177,178] and environmental chemicals[179]. Learning from prokaryotes, these strategies have been developed across cell types. Furthermore, signals can be processed through synthetic gene circuits to achieve Boolean, dynamic responses and sophisticated patterned responses[20,169]. By routing signals to control promoter activity, transcription factor inputs enable simple computation of 'AND' and 'OR' logic that can be built into high-level processing. Synthetic gene networks, such as pulse generators, band-pass detectors, oscillators and toggle switches, convert input signals into temporally defined responses that can be used to time cellular processes and deliver molecules on a defined schedule[171,180,181]. Advances in engineering the secretion of proteins provide an important tool that will accelerate the types of biohybrid systems[163–166]. Importantly, for stability, biohybrid devices will require synthetic population control systems that support homeostasis of cell numbers in the device and prevent overgrowth[182].

Although the ability to synthesize and construct genetic programmes has massively increased since the early 2000s, forward design remains challenging. In particular, circuits with greater numbers of parts and more complex designs are fragile. Coupling through resource sharing, physical coupling and metabolic burdening can lead to emergent behaviours that diminish performance. Simple, single-transcript and single-locus designs can reduce variability and improve predictability[183]. Designs that minimally impact native systems, account for emergent coupling and buffer interactions between modules can improve designs[183–186]. However, integration of genetic programmes for stable cellular engineering remains a challenge[187–189]. Integration into the genome requires consideration of the influence of the native genome on component and circuit behaviour[183,189]. As our understanding of the molecular regulation of the native genome expands, our ability to select locations in the genome and parts that optimize circuit function will improve the robustness of forward design and cellular engineering[151,190,191]. Alternatively, screening for circuit functions represents a useful framework that can be made more powerful as library sizes reach the scale for machine learning-guided design[192].

(continued from previous page)



Box Fig. 1 | Cell engineering overview and functions. Cells are engineered with transgenic cargoes that are processed through the central dogma via transcription and translation[170,193]. Individual components or parts dictate the kinetic rates (k) of transcription, translation, degradation and transport. Varying these rates by design supports complex signal processing[168,169]. Each step in the process of gene expression provides an intervention point for building a genetic sensor. By integrating multiple parts into devices, such as hybrid synthetic promoters, logic functions can enable cells to respond to specific combinations of inputs with defined output matrices[169]. Circuits can do complex signal processing to decode transient from sustained signalling via persistence detectors and pulse detectors. Biohybrid devices can benefit from synthetic population control that can support maintenance of the culture without loss or overgrowth[182,194]. Ideally, biohybrid devices can autonomously deliver signals using clock-like functions of transcriptional oscillators[195,196]. Combining these molecular tools and systems will allow biohybrid devices to receive, process and respond to diverse stimuli and support advanced computation.

surface via electrochemistry is a powerful method for transmitting and receiving information. Such an interface can serve as a conduit for information transfer to (from) biological systems and influence downstream biological outcomes from the molecular to the multicellular level[29]. For example, reducing or oxidizing signalling molecules (such as ferrocene, ferricyanide[22] or hydrogen peroxide) activate engineered gene circuits within cells to enable signal transduction across the cellular population with a high degree of spatiotemporal control[30]. The control of gene expression can be further validated using redox-active products such as p-aminophenol[31] and others[32,33]. Furthermore, redox communication can be extended to additional molecular signalling pathways, for example, myriad NADH-responsive enzymes can be regulated via electron transfer, thus linking electronic signals to a vast array of biological processes[29]. Transmission of information from electrodes into genetic pathways can also be achieved through electrochemically responsive variants of CRISPR[33,34]. For example, redox-activated promoters, like SoxS, can drive the production of guide RNAs that direct the CRISPR to edit specific sites in the genome. In this way, electrical signals can be converted into changes in the genome that can amplify the effects of the electrical input signals. Finally, living or interactive materials, such as catechol-conjugated redox-active hydrogel films, can serve as a platform to bridge electrodes and redox signalling in bacterial systems; interestingly, they can also be assembled electrochemically and modified in real time[35].

As a faster alternative to electrochemical sensing of synthesized proteins, microbes can be engineered to produce electrical currents via direct electron transfer between proteins on the surface of the cell and the electrode. In this way, bioelectronic cell-based sensors can avoid the latency of protein synthesis to provide near real-time sensing within minutes of exposure to an analyte[36]. Cells can also be engineered to produce functional materials that support electrical coupling between cell assemblies, providing another method for synthetic biology to induce fast electronic signalling through cell networks[37,38].

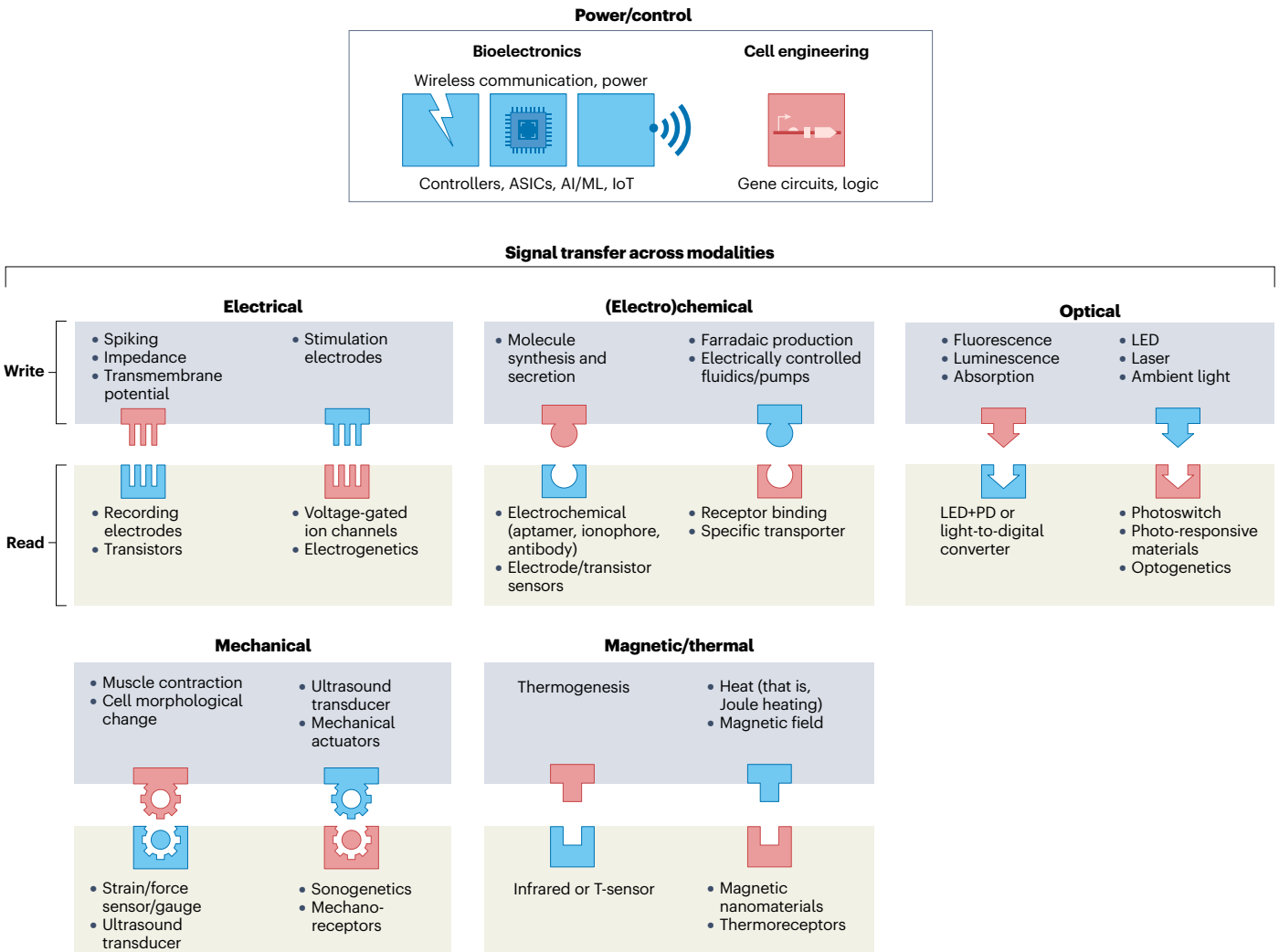


Fig. 2 | Toolbox of biohybrid building blocks (biohybricks). Top, intra-domain components related to power and/or control (that is, microcontrollers and gene circuits). Bottom, inter-domain components that facilitate communication between cell-engineered and bioelectronic components. Input 'read' and output 'write' modalities are shaded in beige and grey, respectively. Cell-based modalities are red and bioelectronic modalities are blue. ASIC, application-specific integrated circuit; AI/ML, artificial intelligence and/or machine learning; IoT, Internet of Things; LED, light-emitting diode; PD, photodiode.

Optical reading and writing

Optical methods to read and write can achieve lower latencies compared to electrochemical methods and improved cell-type specificity compared to direct electrical methods. This cell-type specificity is most easily achieved using 'optogenetic' techniques, whereby light-sensitive proteins are expressed in the cell membrane[39,40]. This method typically involves genetic modification and integration of transmembrane protein complexes (that is, ion channels, pumps or G protein-coupled receptors) that incorporate photoresponsive chromophores that impart light responsivity. Light can also activate cells without genetic manipulation with infrared light[41] or via materials that convert incident photons into heat[42], molecular motion[43], ultrasound[44] or electric fields[45]. Techniques that do not require any genetic manipulation of the host cells might be more suitable for in vivo or clinical applications that lack well-developed transgenic tools. However, these methods lack the cell-type specificity possible with optogenetics.

Optical readout can also be achieved with genetic manipulation of the cells or with small-molecule indicators of calcium, voltage or other signalling molecules[46,47]. The majority of these optical readout technologies rely on measuring fluorescence that changes based on voltage or binding to analytes like calcium or neurotransmitters by using a semiconductor-based photodetector. One of the major challenges with fluorescence is the need for excitation light (that is, from a laser or light-emitting diode (LED)), which can produce cytotoxicity over long periods of time and require high-fidelity filtering to separate the emitted from the excitation light. Bioluminescence has been used as an alternative optical readout, which obviates the need for excitation light because cell activity can be encoded directly into light emitted by the cell[48–50]. However, bioluminescence is rarely used as an optical readout owing to difficulties in delivering the substrate (such as coelenterazine) that is cleaved by the cells to produce light, and difficulty in detecting the low light levels that are produced compared to ambient light. A genetically encoded bioluminescence substrate and improvements in substrate delivery and brightness would enhance the adoption of bioluminescence as an optical readout technology. Alternatively, indicators that convert cell activity into changes in absorption or scattering could offer other non-fluorescent optical methods to measure cell activity.

Mechanical reading and writing

Mechanical activation of cell activity can achieve relatively fast latencies when compared to chemical methods by activating mechanoreceptors on cells but typically requires direct physical contact with the cells[51]. A quasi-remote version of mechanical writing can be achieved with magnetomechanical activation. With this approach, a magnetic field can displace nanoparticles or microparticles in cells or surrounding extracellular matrices that in turn activate cell mechanoreceptors[52–54]. Mechanical forces can also be used for reading cell activity; for example, mechanical actuation of contractile tissues can be used to communicate a response to a stimulus or to actuate miniature robotic biohybrid devices[55,56].

As a subset of mechanical approaches, ultrasound waves can also be used to activate mechanoreceptors and can often achieve deeper penetration compared to direct physical contact, optical illumination or electrical stimulation. Some cells, such as neurons and glia, respond endogenously to ultrasound via activation of an ensemble of mechanoreceptors[57], and ultrasound sensitivity has been observed in both vertebrates[58] and invertebrates[59].

In addition to stimulation, ultrasound can be used to record cell activity based on calcium-dependent contrast from gas vesicles[60]. Microbes and mammalian cells can be genetically modified to express the ensemble proteins that create these gas vesicles[61]. Furthermore, these vesicles can be engineered to produce ultrasound contrast that depends on the local calcium concentration. In this way, ultrasound can be used as an effective deep-tissue readout of genetically modified cell activity. Both ultrasound actuation and imaging approaches will benefit from advances in wearable, adhesive and implantable ultrasound systems[62,63]. Such systems rely on piezoelectric elements that emit sound waves into tissues (2–18 MHz) and/or measure the reflected waves to form images.

Other modalities of writing

Typically reserved for invertebrate small-animal models, thermoreceptors can be overexpressed in specific cells to enable activation by heating or cooling the tissue or animal. Alternatively, temperature can be used to control membrane localization of genetically encoded proteins[64]. One of the main challenges of 'thermogenetics'[64] is that there are often endogenous thermoreceptors and other temperature-sensitive chemical processes that limit its specificity. One way to improve thermogenetic activation is to use magnetic fields to heat magnetic nanoparticles that can be injected into specific regions of the tissue. The advantage of magnetothermal activation is that the localized rapid heating can be much more targeted than heating the entire tissue. Magnetothermal activation of mammalian cells can stimulate cell activity with latencies on the order of seconds[65,66]. This latency is driven by the need for nanoparticles to reach the threshold temperature of the TRPV1 thermoreceptors in the target cells. Alternatively, rate-dependent thermoreceptors (TRPA1) that are activated by fast changes in temperature produce much faster cell activation with behavioural latencies of 0.5 s (ref. 67) and can be activated with less change in local temperature when compared to TRPV1 channels. These studies were performed in *Drosophila*, which have rate-dependent TRPA1 channels. Applying similar methods in mammals could improve the response time for magnetothermal activation.

Magnetoelectric materials can, in principle, activate cells more rapidly than magnetothermal methods because voltage-gated channels should respond to depolarization within milliseconds. Several approaches to use magnetoelectric nanoparticles have indeed shown the ability to activate cells with an applied magnetic field but most studies show latencies of several seconds[68,69]. This relatively long latency is the result of weak electric fields produced by nanoparticles, which do not drive sufficient depolarization to activate electrically excitable cells. To increase the electric field, magnetoelectric materials can be driven at their resonance frequency; however, this frequency is typically far above the cutoff frequency of the cell membrane, making it ineffective for stimulation. A self-rectifying magnetoelectric 'metamaterial' can overcome the mismatch between the cell membrane response and the high-frequency resonant mode to achieve millisecond latencies[70].

Key challenges towards biohybrid integration

Protection from fouling and fibrosis

Implanted biohybrid devices can trigger a cascade of host-mediated immune responses, which results in the fibrous encapsulation of the implant and compromises device function and durability[71]. These foreign body reactions occur on the order of days to weeks after implantation and represent a critical hurdle to the functional activity

of chronically implanted devices. Thus, mitigation strategies must be integrated into the design of biohybrid devices to ensure protection against fibrosis. There are many ways to prevent fibrosis of implants[72] some of which are particularly relevant for biohybrid or bioelectric implants. For example, surface modification enables implants to evade the immune system and resist non-specific adhesion of biomolecules; this approach involves the application of biocompatible coatings, such as zwitterionic modifications, on the encapsulating hydrogels and electronic components. Large libraries of small molecules have been screened and small-molecule coatings, such as Z4-A10, have been identified to prevent the fibrotic response to implanted encapsulated cells[73]. This method prevented fibrosis and protected the viability of cells for at least 6 months in the immune-competent C57BL/6J STZ-induced diabetic mouse model[74]. Release of cytokines from the implant can also prevent the fibrotic response from sequestering implants. For example, IL-2 release from an encapsulated cell implant in the intraperitoneal space of C57BL/6J prevented fibrosis for 2 weeks[75]. Cells can also be engineered to secrete other immunomodulatory molecules, such as IL-10, IL-33 and IL-35, to limit host immune recognition, suppress inflammation and promote engraftment[76].

Co-implantation of crystalized anti-inflammatory drugs, such as GW2580, a colony-stimulating factor 1 receptor inhibitor, has also prevented fibrosis of electrode-based glucose sensors[77]. Other approaches showing promise in reducing implant fibrosis include modifying device topography[78], surface charge[79] and stiffness[72,80].

Chronic real-time biosensing in complex in vivo environments can be hampered by protein adsorption and host immune responses leading to reactive oxygen species and decay by tissue remodelling factors (that is, degradation enzymes)[27]. Surface modifications can also help reduce inflammation[81], repel protein adsorption[82] and release anti-inflammatory molecules to reduce signal decay and maintain the accurate performance of chronic sensors[83]. In the case of electrical stimulation, biofouling can be compensated for by increasing the amplitude of the stimulator but this might approach the limits of safety or practicality. Thus, both electronics and biological elements can each be engineered to overcome challenges like fibrosis and/or fouling.

Supporting cell viability and function

High-density cell therapies are essential for compact therapeutic devices. Encapsulation allows cells to survive at 6,000–40,000 cells mm^{-3} for chronic disease treatment, for example, diabetes and psoriasis[84,85]. Yet, preserving cell function over time is challenging not only due to host immune responses but also due to lack of nutrients and oxygen, with oxygen being regarded as the limiting factor supporting cell viability and potency[86]. In native tissues, each cell is within ~100 µm from a blood capillary to allow adequate oxygen supply, which is limited by the mass diffusion of oxygen[86]. Transplanted exogenous cells or tissue require the formation of new blood vessels or supplemental oxygenation[87]. Resilience to hypoxia can be engineered into cells using genetic constructs. Human cells can naturally adapt to hypoxic environments through the regulation of hypoxia-inducible factors (HIFs), which can be tuned to achieve cell resilience[88]. These master regulators can be leveraged to modulate critical cell functions such as metabolism, immunogenicity and cell plasticity. The same pathways can also be harnessed to promote the release of pro-angiogenic molecules like VEGF and suppress apoptosis pathways to protect against cell death[89,90].

Oxygen deficiency in the transplanted cells is caused by (1) insufficient oxygen tension at the implantation site, (2) innate large oxygen consumption of cells (that is, metabolic demand), (3) high cell density and (4) additional barriers to oxygen diffusion (such as membranes or formation of encapsulating fibrotic tissue). To address the hypoxic stress, various strategies have been investigated to enhance exogenous oxygen delivery. Active methods involve oxygen release through an externally controllable mechanism, for example, delivery of gaseous oxygen to transplanted cells such as islets[91]. Passive methods rely on gradual release of oxygen through unregulated or self-regulated mechanisms, such as engineered platforms, to increase the oxygen exchange with the implantation environment[92] or release of oxygen from metal peroxides[93–95]. Although these strategies can support transplanted cells, they are limited in the control of oxygen release and lifetime of available oxygen supply and thus have limited supported cell density. Electrochemical water electrolysis for oxygen production is a promising active approach for providing oxygen to cells[96,97]. In electrolysis, oxygen is produced by splitting water at an electrode (anode) interface while hydrogen is produced at another electrode (cathode). However, its demonstration in vivo has been limited due to improper materials selection for water splitting in neutral pH, the use of bulky or complex electronics, and a limited power budget[91,97–99]. New bioelectronic electrocatalytic oxygenators for sustaining high-density (up to 60,000 cells mm^{-3}) implanted cells in vitro and in vivo were demonstrated, opening new avenues in bioelectronic cell support[100,101]. Oxygen generation is achieved through electrocatalysis of water and precisely regulated using either battery-powered or wireless, battery-free power transfer. The choice of electrocatalyst, such as nanostructured iridium oxide, which is a highly active, biocompatible electrocatalyst for water oxidation, is critical for efficient and selective oxygen production[100,101]. Other cell engineering strategies can also be leveraged to survive transient hypoxic conditions and support a more robust function of devices[90]. For example, although metabolic engineering has generally focused on optimizing the production of a desired product, these optimizations often minimize central metabolism. Thus, minimizing metabolism might improve the performance of engineered cells and stabilize them during short periods of low oxygenation as well as limiting the requirements of nutrient supply and waste removal[102].

Manufacturing and regulatory considerations

Just as the choice of biomaterials and coatings can influence cell and device function and longevity, so too will the choice of cell chassis for the biological system. Even the high-level choice of bacterial versus mammalian cells substantially changes paths of device development, manufacturing and supply chain, and regulatory approvals. Owing to their lower division rates and rates of mutation, mammalian cells have lower rates of genetic drift, which can corrupt desired function. The hardiness and rapid division rate of bacteria makes them adaptable and responsive to harsh and variable conditions, yet these same features allow bacteria to more rapidly mutate encoded gene circuits, which can destroy their function. Across the human body, populations of bacteria are well tolerated within their specialized niches such as the gut. However, the risk of sepsis in the event of the release of microbes into the blood makes bacteria a riskier cell type for in vivo applications beyond the gut. Even within mammalian cells, HEK cells are often used in various applications owing to their resilience, ease of genetic manipulation and response to transgenic programmes[103,104]. Nevertheless, cell therapy products are emerging as an important class of medicines with more than 35 FDA-approved cellular therapies on the market today[105].

However, moving beyond a proof-of-concept lab demonstration to deploying or translating a biohybrid device, new barriers are ever present. Although good manufacturing and good lab practice and standards are well established in the biomedical device and cell therapy communities separately, their integration within a single device is less straightforward. For example, the point in an assembly process at which the living component is integrated with the abiotic materials or electronics needs to be considered. Although the hardware can be sterilized and packaged for long-term storage, once the living component is added, storage and shipment protocols relevant to cells must be implemented without the opportunity to re-sterilize the device before deployment. As such, the lifetime of the electronics, encapsulation, coatings and biomaterials must therefore consider the assembly and storage process, where bioelectronic-only devices are largely engineered for their time after deployment (in the body, for example). Alternatively, the living component and hardware could be integrated at the point of application, posing additional risks in quality control.

These considerations hinder approvals for biohybrid devices for biomedical or environmental applications. Such devices might be viewed as a combination product by regulatory bodies, which could require both investigational device exemption and investigational new drug approvals, with one taking the lead depending on the primary mode of action of the device. There are few predicate 'living' devices that combine bioelectronic sensing and/or actuation with exogenous living cells, meaning that each new product is shaping the approvals landscape.

'Device' implementation examples

With technology in place for supporting, activating and/or reading from engineered living constructs, key examples of biohybrid bioelectronics devices have emerged. Although many examples are rudimentary and might not represent a closed system, they show potential for such approaches across domains.

Biohybrid mechanical actuation

There has been a considerable focus on designing and deploying engineered cell-based actuators motivated by their increased scalability, adaptability and energy efficiency compared to traditional actuators[106]. Robust bioelectronic interfaces that precisely control contractile tissue activity and integrate closed-loop sensory feedback would enable deploying bioactuators in real-world applications in medicine and robotics.

Pacing and on/off control of cardiac and skeletal muscle-based actuators has primarily been achieved via electrical stimulation or, in the case of optogenetic cell-based systems, light stimulation. Although these approaches enable spatiotemporal control of muscle actuation, they have largely been implemented as stationary arrays of rigid electrodes, LEDs or optical fibres that do not adapt their stimulation parameters to accommodate changing muscle position, thus limiting their potential for untethered functionality[55,107–109]. New methods for manufacturing light and battery-free electronics that can be stably attached to actuating tissues and remotely controlled via radio frequency power sources have, however, showcased the transformative potential for bioelectronic innovations in bioactuators[110,111] (Fig. 3a).

Equally as important as bioelectronic control of bioactuators but less explored are methods to enable real-time functional readout from contractile tissues[112]. Because triggerable actuation is the primary function of bioactuators, most efforts to integrate bioelectronic sensors with cardiac and skeletal muscle tissues have focused on precise displacement readout via compliant strain sensors[113,114]. Although important, displacement-centred sensory readouts neglect emerging understanding that muscle actuation is coupled with its function as a secretory organ[115,116]. Future work targeted at integrating biochemical sensing with bioactuators could thus prove equally important for monitoring muscle function in real time.

Closed-loop feedback of bioactuators that couples the output of sensors to the input of upstream controllers has yet to be explored in depth for in vitro systems targeted for applications in robotics. However, monitoring tissue movement and using this information to modulate upstream neural control of muscle has been demonstrated in vivo for bioelectronic applications in prosthetics[117], providing an ample knowledge base to drive future advances in closed-loop feedback control of engineered bioactuators.

Notably, bioelectronic interfacing with bioactuators has largely focused on direct control of and sensory readout from muscle rather than monitoring and modulation of upstream neuronal networks. Single-cell level and lower power control of bioactuators would require complete functional innervation of contractile tissue and bioelectronic interfacing with motor and sensory neurons. Although bioelectronic control and monitoring of neurons have been extensively explored and characterized[118,119], the challenge of innervating millimeter-scale 3D tissues and forming functional motor control circuits remains unsolved[8,120], presenting an open future opportunity in this field.

Biohybrid 'living pharmacies'

Using the natural machinery of the cell, biomolecule production can be initiated or controlled (using the multiple modalities described above) for wide-ranging applications in cell-based therapeutics.

Light-stimulated therapy production. Optogenetics enables precise control of cellular function through light stimuli[121]. This method allows cells to respond to specific wavelengths of light, triggering the synthesis and secretion of therapeutic agents. Owing to the temporal sensitivity of diabetes therapy delivery, the effectiveness of optogenetic systems was first tested for the secretion of a short variant of human GLP1 and insulin in response to light stimulation[122–124]. Light-induction of various cancer immunotherapies has also been demonstrated to reduce tumour burden in a mouse cancer[125].

Owing to the wide range of wavelengths of light that can stimulate optogenetic systems, multiple systems can act independently of each other. Three different optogenetic systems, activated by blue, red or UV-B light, were able to be individually activated without cross-activation[126]. This feature is potentially useful in low-space settings, such as in a medical implant targeting the regulated production of multiple biomolecules independently.

Although most work in engineering cells for controlled therapeutic secretion is focused on mammalian cells, bacterial cells have also been used in some cases. For example, certain anaerobic bacteria can localize to a tumour microenvironment, and by using a light-activated optogenetic kill switch, the bacteria can be forced to lyse and release anti-tumour factors to treat the cancer to which they are localized[127]. Light delivery in these cases depends on the desired wavelength and spatial localization, ranging from transcutaneous illumination to fibres or wireless LEDs.

Electrical or electrochemical therapy production. Electrogenetics focuses on engineering cells to secrete therapies in response to electrical stimulation, a concept that is relatively new compared

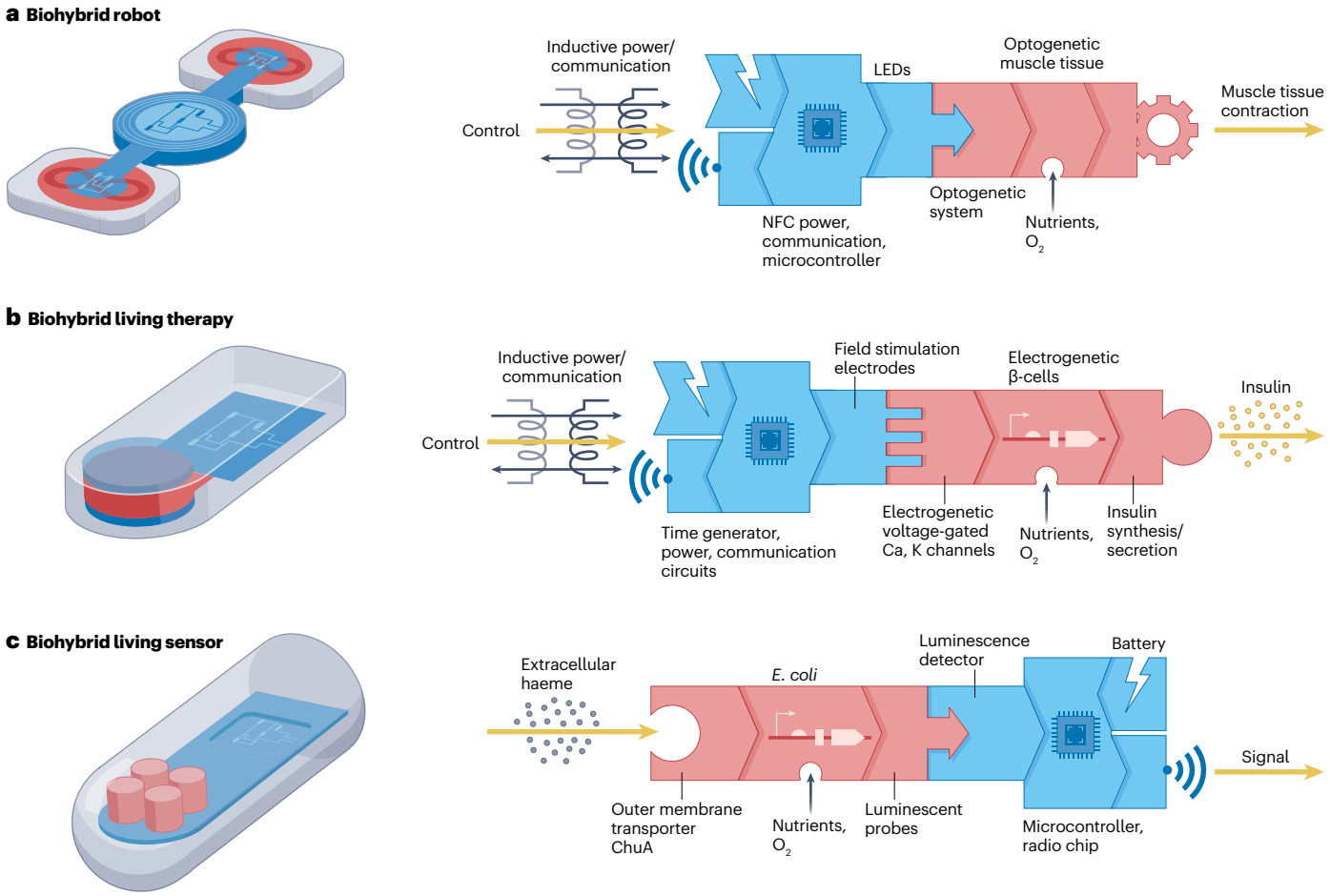


Fig. 3 | Examples of biohybrid systems across modalities. Rough device structures are shown on the left, highlighting where bioelectronic and engineered cellular components reside. Biohybrick representation of biotic (engineered cells, red) and abiotic (bioelectronic, blue) components used to build the systems are shown on the right. **a**, Wireless platform for a steerable swimming biohybrid robot concept using LED actuation of optogenetic muscle tissues[110,111]. **b**, Biohybrid living therapy concept leveraging electrogenetic β-cells to deliver insulin[128]. **c**, Wireless gut-born biohybrid living sensor leveraging *Escherichia coli*-engineered luminescence on sensing of extracellular haeme[152]. LED, light-emitting diode; NFC, near-field communication.

to optogenetics, and allows for unique methods of stimulation when implanted. For example, voltage-activated calcium channels can be used to activate the nuclear factor of activated T cells (NFAT) signalling pathway for transgene expression and induce the secretion of insulin-containing vesicles from engineered β-cells[128]. These engineered cells were encapsulated in an implant that was wirelessly powered by a field generator to stimulate the cells inside mice (Fig. 3b). A second electrogenetic system, named DC-actuated regulation technology, is activated via endogenous generation of reactive oxygen species caused by direct cell stimulation with a metallic electrode[129]. After subcutaneous implantation, these cells can be transdermally simulated with acupuncture needle electrodes, where the electrodes are inserted through the skin at opposite sides of the implanted cell and a voltage applied to them. Electrogenetics benefits from a simple setup for stimulation (that is, two electrodes) compared to the other methods of transgene control. Moreover, it has lower power consumption compared to optical stimulation approaches.

Electrogenetics can also actuate cells by longer-distance mechanisms; for example, redox reactions produce reduced oxygen that cells can sense and respond to, which can be used to control the cells in vitro[130]. This method can signal at a distance from the device initiating the electrical signalling unlike direct-electrode stimulation[30].

Mechanogenetic and magnetogenetic therapy production. Mechanogenetics and magnetogenetics provide flexible platforms for precise cellular control in therapeutic contexts. Mechanogenetic therapy induction has been used to activate the expression of chimaeric antigen receptors in response to ultrasound stimulation in Jurkat T cells in vitro[131]. Similarly, heating nanoparticles induces transgene expression of heat-sensitive ion channels in mammalian cells to activate the NFAT signalling pathway, which has been used for controlled expression of insulin for glucose correction in mice[132].

Biohybrid cell-based chemical sensors

Cells are phenomenal sensors of their local environment. Although sensory organs are often highlighted for their ability to respond to biochemical and biophysical cues outside an organism, every cell is riddled with receptors that initiate biochemical signalling cascades that

can be leveraged for biochemical sensing and readout. By harvesting native components and engineering them into modular genetic parts, cells can also be engineered to respond to diverse physiochemical cues, including light[133–136], temperature[64,137], pH[138], mechanical stimuli[131,139,140], small molecules[141–143], synthetic and native proteins[144–146], and cell–cell interactions[147–149]. Genetically encoded sensor molecules can direct external stimuli into native and synthetic protein and transcriptional networks to compute and produce programmed cell responses[150,151]. They also allow for renewable function as these sensor molecules are regenerated through gene expression, thus offering the potential for continuous sensing. However, continuous real-time sensing still faces many challenges, in that the engineered cells will respond to an initial cue and propagate the desired genetic circuit, but subsequent changes in the same cue will not be sensed by that same cell and will not alter the cell response[152,153]. By integrating sensing and computation of signals, engineered cells augment electronic devices to serve as modular, programmable and low-resource systems that expand the capabilities of biohybrid systems[154].

Cell-based health diagnostics. Owing to their natural abundance in the gastrointestinal tract, bacteria are a promising chassis for sensing markers of stomach and bowel disease. Engineered bacterial strains encapsulated within ingestible devices can monitor gastrointestinal health and detect colonic inflammation through luminescent and fluorescent outputs[152,155,156]. Example implementations use microelectronic luminescence sensors and wireless communication to transmit information about sensed biochemicals such as extracellular haeme[152] (Fig. 3c). Mammalian cells have also been engineered for non-gastrointestinal sensing applications, for example, to express reporter proteins in response to physiological cues, such as hyperosmolarity, and disease markers such as VEGF, offering potential avenues for health diagnostics and therapeutic monitoring[157,158].

Sensors for environmental chemical monitoring. Bacteria have also been engineered to sense environmental chemicals by detecting pollutants and heavy metal contamination. Genetic manipulation enables these microbial sensors to selectively respond to target compounds, facilitating the detection of toluene, heavy metals and polycyclic aromatic hydrocarbons in diverse environmental settings[159–161]. These types of bacterial sensors can be encapsulated in biomaterials to facilitate environmental sensing[36]. Such living sensors can similarly be probed with optical or electrical readouts.

Outlook

The union of engineered biological systems with bioelectronic components presents many opportunities in health, robotics and the environment. By harnessing the parallel developments in genome editing, biomaterials and tissue engineering, and bioelectronics, living biohybrid bioelectronics can synergistically combine their strengths. Current demonstrations are either few or limited to proof-of-concept demonstrations, still lacking in key metrics that would enable their translation. For example, questions about how long a living biohybrid bioelectronic device can last remain unanswered. Although short-term biomedical devices can have relevance in some applications (that is, anti-virals, contraceptives or oncotherapies), many chronic conditions require long implementation, motivating deeper studies of resilience and lifetime control in such systems.

Another key limitation of biohybrid bioelectronics is that pioneering examples in this domain have largely focused on tissues derived from single-cell types for single purposes. By contrast, most natural biological systems rely on multicellular assemblies of diverse specialized cell types to generate complex and adaptive functional behaviours[162]. Biohybrid robotics, as one use case, have thus far leveraged simple actuator designs limited to the sterile environment of a petri dish. How neural control and sensory feedback can be integrated in biohybrid robots to achieve more complex autonomous functionalities and robust adaption to unpredictable conditions remains to be established. Multiple cell types can also benefit biohybrid sensors and therapies: support cells can be integrated to protect against inflammation or fibrosis, report on cell state, or enable multiple co-located cell types for multiplexed actuation or sensing.

Finally, across applications, standalone wireless devices that can perform multiple, multimodal functions and communicate wirelessly require continued advances in power efficiency. This must include advances in wireless, long-range power transfer, or improvements in the capacity and miniaturization of on-board batteries.

The next frontier in living biohybrid bioelectronics will be responsive and evolvable. Such functionality will lean on both the living and bioelectronic components. For example, internal feedback will allow for adaptive control: enteroception or proprioception via mechanical sensing in biohybrid robotics will provide feedback for controls and preserve the longevity of muscle tissue, avoiding fatigue. Living or abiotic chemical sensors will enable precision and personalized dosing for living therapeutics. This will enable fine dose control for therapies with narrow therapeutic ranges. Furthermore, external feedback will enable sensors to modulate production due to toxicity biomarkers or to allow for timed therapy production when needed or when most efficacious. External feedback will empower a biohybrid robot to explore or seek certain cues in both biological and environmental settings. Finally, dynamic reprogramming (both biological and bioelectronic) will enable a device to evolve over time towards the needs of the targeted application or as a means for device self-preservation.

These advances will require expertise in synthetic biology, biomaterials and bioelectronics to continue their rapid advances. More importantly, they require co-design and convergence research. The biotic and abiotic components cannot be designed separately and brought together. Each component has an outsized effect that influences the design choices of other components. Each design metric or constraint is dependent on the desired application and outcome, including location or tissue type, cell types, densities, and duration of operation. Power requirements influence the size and heating of a device, thus influencing the number of cells or their viability. Oxygen tension influences fluorescent protein maturation and hence optoelectronic readouts for cell-based sensing. By bringing these communities together, living biohybrid bioelectronics will lead to advances in regulated therapies, diagnostics, regenerative engineering, robotics, computation, environmental remediation, and energy storage or production.

Published online: 07 January 2025

References

1. Selberg, J., Gomez, M. & Rolandi, M. The potential for convergence between synthetic biology and bioelectronics. *Cell Syst.* **7**, 231–244 (2018).
2. Fischbach, M. A., Bluestone, J. A. & Lim, W. A. Cell-based therapeutics: the next pillar of medicine. *Sci. Transl. Med.* **5**, 179ps7 (2013).
3. Duan, F. & March, J. C. Engineered bacterial communication prevents Vibrio cholerae virulence in an infant mouse model. *Proc. Natl Acad. Sci. USA* **107**, 11260–11264 (2010).
4. Riglar, D. T. et al. Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation. *Nat. Biotechnol.* **35**, 653–658 (2017).

5. Courbet, A., Endy, D., Renard, E., Molina, F. & Bonnet, J. Detection of pathological biomarkers in human clinical samples via amplifying genetic switches and logic gates. *Sci. Transl. Med.* **7**, 289ra83 (2015).
6. Roggo, C. & van der Meer, J. R. Miniaturized and integrated whole cell living bacterial sensors in field applicable autonomous devices. *Curr. Opin. Biotechnol.* **45**, 24–33 (2017).
7. Duan, F. F., Liu, J. H. & March, J. C. Engineered commensal bacteria reprogram intestinal cells into glucose-responsive insulin-secreting cells for the treatment of diabetes. *Diabetes* **64**, 1794–1803 (2015).
8. Raman, R. Engineered neuromuscular actuators for medicine, meat, and machines. *MRS Bull.* **46**, 522–533 (2021).
9. Peterman, E. L., Ploessl, D. & Galloway, K. E. Accelerating diverse cell-based therapies through scalable design. *Annu. Rev. Chem. Biomol. Eng.* **15**, 267–292 (2024).
10. Blanch-Asensio, A. et al. Generation of AAVS1 and CLYBL STRAIGHT-IN v2 acceptor human iPSC lines for integrating DNA payloads. *Stem Cell Res.* **66**, 102991 (2023).
11. Yarnall, M. T. N. et al. Drag-and-drop genome insertion of large sequences without double-strand DNA cleavage using CRISPR-directed integrases. *Nat. Biotechnol.* **41**, 500–512 (2023).
12. Pandey, S. et al. Efficient site-specific integration of large genes in mammalian cells via continuously evolved recombinases and prime editing. *Nat. Biomed. Eng*. https://doi.org/10.1038/s41551-024-01227-1 (2024).
13. Song, Y. et al. 3D-printed epifluidic electronic skin for machine learning–powered multimodal health surveillance. *Sci. Adv.* **9**, eadi6492 (2023).
14. Wang, W. et al. Neuromorphic sensorimotor loop embodied by monolithically integrated, low-voltage, soft e-skin. *Science* **380**, 735–742 (2023).
15. Song, E., Li, J. & Rogers, J. A. Barrier materials for flexible bioelectronic implants with chronic stability—current approaches and future directions. *APL Mater.* **7**, 050902 (2019).
16. Nair, V. et al. Miniature battery-free bioelectronics. *Science* **382**, eabn4732 (2023).
17. Adee, S. *We Are Electric* (Hachette Books, 2022).
18. Park, J. et al. Screening fluorescent voltage indicators with spontaneously spiking HEK cells. *PLoS One* **8**, e85221 (2013).
19. McNamara, H. M. et al. Bioelectrical domain walls in homogeneous tissues. *Nat. Phys.* **16**, 357–364 (2020).
20. Mansouri, M. & Fussenegger, M. Electrogenetics: bridging synthetic biology and electronics to remotely control the behavior of mammalian designer cells. *Curr. Opin. Chem. Biol.* **68**, 102151 (2022).
21. Weber, W. et al. A synthetic mammalian electro-genetic transcription circuit. *Nucleic Acids Res.* **37**, e33 (2009).
22. Tschirhart, T. et al. Electronic control of gene expression and cell behaviour in Escherichia coli through redox signalling. *Nat. Commun.* **8**, 14030 (2017).
23. Wang, S., Aljirafi, F. O., Payne, G. F. & Bentley, W. E. Excite the unexcitable: engineering cells and redox signaling for targeted bioelectronic control. *Curr. Opin. Biotechnol.* **85**, 103052 (2024).
24. Rivnay, J., Wang, H., Fenno, L., Deisseroth, K. & Malliaras, G. G. Next-generation probes, particles, and proteins for neural interfacing. *Sci. Adv.* **3**, e1601649 (2017).
25. Castagnola, E., Garg, R., Rastogi, S. K., Cohen-Karni, T. & Cui, X. T. 3D fuzzy graphene microelectrode array for dopamine sensing at sub-cellular spatial resolution. *Biosens. Bioelectron.* **191**, 113440 (2021).
26. Patel, P. R. et al. High density carbon fiber arrays for chronic electrophysiology, fast scan cyclic voltammetry, and correlative anatomy. *J. Neural Eng.* **17**, 056029 (2020).
27. Flynn, C. D. et al. Biomolecular sensors for advanced physiological monitoring. *Nat. Rev. Bioeng.* **1**, 560–575 (2023).
28. Wu, J., Liu, H., Chen, W., Ma, B. & Ju, H. Device integration of electrochemical biosensors. *Nat. Rev. Bioeng.* **1**, 346–360 (2023).
29. VanArsdale, E., Pitzer, J., Payne, G. F. & Bentley, W. E. Redox electrochemistry to interrogate and control biomolecular communication. *iScience* **23**, 101545 (2020).
30. Wang, S. et al. Redox-enabled electronic interrogation and feedback control of hierarchical and networked biological systems. *Nat. Commun.* **14**, 8514 (2023).
31. Terrell, J. L. et al. Bioelectronic control of a microbial community using surface-assembled electrogenetic cells to route signals. *Nat. Nanotechnol.* **16**, 688–697 (2021).
32. VanArsdale, E. et al. A coculture based tyrosine-tyrosinase electrochemical gene circuit for connecting cellular communication with electronic networks. *ACS Synth. Biol.* **9**, 1117–1128 (2020).
33. VanArsdale, E. et al. Redox-based synthetic biology enables electrochemical detection of the herbicides dicamba and roundup via rewired *Escherichia coli*. *ACS Sens.* **4**, 1180–1184 (2019).
34. Bhokisham, N. et al. A redox-based electrogenetic CRISPR system to connect with and control biological information networks. *Nat. Commun.* **11**, 2427 (2020).
This article shows a version of CRISPR that converts an electronically generated redox signal into protein synthesis taking a step towards electronic control of gene expression.
35. Li, J. et al. Interactive materials for bidirectional redox-based communication. *Adv. Mater.* **33**, e2007758 (2021).
36. Atkinson, J. T. et al. Real-time bioelectronic sensing of environmental contaminants. *Nature* **611**, 548–553 (2022).
37. Liu, J. et al. Genetically targeted chemical assembly of functional materials in living cells, tissues, and animals. *Science* **367**, 1372–1376 (2020).
38. Zhang, A. et al. Genetically targeted chemical assembly of polymers specifically localized extracellularly to surface membranes of living neurons. *Sci. Adv.* **9**, eadi1870 (2023).
39. Deisseroth, K. Optogenetics. *Nat. Methods* **8**, 26–29 (2011).
40. Bansal, A., Shikha, S. & Zhang, Y. Towards translational optogenetics. *Nat. Biomed. Eng.* **7**, 349–369 (2023).
41. Wells, J. et al. Optical stimulation of neural tissue in vivo. *Opt. Lett.* **30**, 504–506 (2005).
42. Kang, H. et al. Thermoplasmonic optical fiber for localized neural stimulation. *ACS Nano* **14**, 11406–11419 (2020).
43. Beckham, J. L. et al. Molecular machines stimulate intercellular calcium waves and cause muscle contraction. *Nat. Nanotechnol.* **18**, 1051–1059 (2023).
44. Shi, L. et al. Non-genetic photoacoustic stimulation of single neurons by a tapered fiber optoacoustic emitter. *Light Sci. Appl.* **10**, 143 (2021).
45. Silverå Ejneby, M. et al. Chronic electrical stimulation of peripheral nerves via deep-red light transduced by an implanted organic photocapacitor. *Nat. Biomed. Eng.* **6**, 741–753 (2022).
46. Peterka, D. S., Takahashi, H. & Yuste, R. Imaging voltage in neurons. *Neuron* **69**, 9–21 (2011).
47. Grienberger, C. & Konnerth, A. Imaging calcium in neurons. *Neuron* **73**, 862–885 (2012).
48. Iwano, S. et al. Single-cell bioluminescence imaging of deep tissue in freely moving animals. *Science* **359**, 935–939 (2018).
49. Naumann, E. A., Kampff, A. R., Prober, D. A., Schier, A. F. & Engert, F. Monitoring neural activity with bioluminescence during natural behavior. *Nat. Neurosci.* **13**, 513–520 (2010).
50. Petersen, E. D. et al. Bioluminescent genetically encoded glutamate indicators for molecular imaging of neuronal activity. *ACS Synth. Biol.* **12**, 2301–2309 (2023).
51. Handler, A. & Ginty, D. D. The mechanosensory neurons of touch and their mechanisms of activation. *Nat. Rev. Neurosci.* **22**, 521–537 (2021).
52. Gregurec, D. et al. Magnetic vortex nanodiscs enable remote magnetomechanical neural stimulation. *ACS Nano* **14**, 8036–8045 (2020).
53. Lee, J. et al. Non-contact long-range magnetic stimulation of mechanosensitive ion channels in freely moving animals. *Nat. Mater.* **20**, 1029–1036 (2021).
54. Rios, B. et al. Mechanically programming anisotropy in engineered muscle with actuating extracellular matrices. *Device* **1**, 100097 (2023).
55. Raman, R. et al. Optogenetic skeletal muscle-powered adaptive biological machines. *Proc. Natl Acad. Sci. USA* **113**, 3497–3502 (2016).
56. Raman, R., Cvetkovic, C. & Bashir, R. A modular approach to the design, fabrication, and characterization of muscle-powered biological machines. *Nat. Protoc.* **12**, 519–533 (2017).
57. Yoo, S., Mittelstein, D. R., Hurt, R. C., Lacroix, J. & Shapiro, M. G. Focused ultrasound excites cortical neurons via mechanosensitive calcium accumulation and ion channel amplification. *Nat. Commun.* **13**, 493 (2022).
58. Tufail, Y., Yoshihiro, A., Pati, S., Li, M. M. & Tyler, W. J. Ultrasonic neuromodulation by brain stimulation with transcranial ultrasound. *Nat. Protoc.* **6**, 1453–1470 (2011).
59. Kubanek, J., Shukla, P., Das, A., Baccus, S. A. & Goodman, M. B. Ultrasound elicits behavioral responses through mechanical effects on neurons and ion channels in a simple nervous system. *J. Neurosci.* **38**, 3081–3091 (2018).
60. Bourdeau, R. W. et al. Acoustic reporter genes for noninvasive imaging of microorganisms in mammalian hosts. *Nature* **553**, 86–90 (2018).
61. Farhadi, A., Ho, G. H., Sawyer, D. P., Bourdeau, R. W. & Shapiro, M. G. Ultrasound imaging of gene expression in mammalian cells. *Science* **365**, 1469–1475 (2019).
62. Hu, H. et al. A wearable cardiac ultrasound imager. *Nature* **613**, 667–675 (2023).
63. Liu, H.-C. et al. Wearable bioadhesive ultrasound shear wave elastography. *Sci. Adv.* **10**, eadk8426 (2024).
64. Benman, W. et al. A temperature-inducible protein module for control of mammalian cell fate. Preprint at *bioRxiv* https://doi.org/10.1101/2024.02.19.581019 (2024).
65. Chen, R., Romero, G., Christiansen, M. G., Mohr, A. & Anikeeva, P. Wireless magnetothermal deep brain stimulation. *Science* **347**, 1477–1480 (2015).
66. Munshi, R. et al. Magnetothermal genetic deep brain stimulation of motor behaviors in awake, freely moving mice. *eLife* **6**, e27069 (2017).
67. Sebesta, C. et al. Subsecond multichannel magnetic control of select neural circuits in freely moving flies. *Nat. Mater.* **21**, 951–958 (2022).
68. Nguyen, T. et al. In vivo wireless brain stimulation via non-invasive and targeted delivery of magnetoelectric nanoparticles. *Neurotherapeutics* **18**, 2091–2106 (2021).
69. Kozielski, K. L. et al. Nonresonant powering of injectable nanoelectrodes enables wireless deep brain stimulation in freely moving mice. *Sci. Adv.* **7**, eabc4189 (2021).
70. Chen, J. C. et al. Self-rectifying magnetoelectric metamaterials for remote neural stimulation and motor function restoration. *Nat. Mater.* **23**, 139–146 (2024).
71. Veiseh, O. & Vegas, A. J. Domesticating the foreign body response: recent advances and applications. *Adv. Drug Deliv. Rev.* **144**, 148–161 (2019).
72. Capuani, S., Malgir, G., Chua, C. Y. X. & Grattoni, A. Advanced strategies to thwart foreign body response to implantable devices. *Bioeng. Transl. Med.* **7**, e10300 (2022).
73. Mukherjee, S. et al. Screening hydrogels for antifibrotic properties by implanting cellularly barcoded alginates in mice and a non-human primate. *Nat. Biomed. Eng.* **7**, 867–886 (2023).
This article highlights the identification and utility of antifibrotic coatings for devices and cell therapies.
74. Vegas, A. J. et al. Long-term glycemic control using polymer-encapsulated human stem cell-derived beta cells in immune-competent mice. *Nat. Med.* **22**, 306–311 (2016).
75. Nash, A. M. et al. Clinically translatable cytokine delivery platform for eradication of intraperitoneal tumors. *Sci. Adv.* **8**, eabm1032 (2022).
76. Nash, A., Lokhorst, N. & Veiseh, O. Localized immunomodulation technologies to enable cellular and organoid transplantation. *Trends Mol. Med.* **29**, 635–645 (2023).

77. Farah, S. et al. Long-term implant fibrosis prevention in rodents and non-human primates using crystallized drug formulations. *Nat. Mater.* **18**, 892–904 (2019).
78. Doloff, J. C. et al. The surface topography of silicone breast implants mediates the foreign body response in mice, rabbits and humans. *Nat. Biomed. Eng.* **5**, 1115–1130 (2021).
79. Zhang, L. et al. Zwitterionic hydrogels implanted in mice resist the foreign-body reaction. *Nat. Biotechnol.* **31**, 553–556 (2013).
80. Noskovicova, N. et al. Suppression of the fibrotic encapsulation of silicone implants by inhibiting the mechanical activation of pro-fibrotic TGF-β. *Nat. Biomed. Eng.* **5**, 1437–1456 (2021).
81. Xie, X. et al. Reduction of measurement noise in a continuous glucose monitor by coating the sensor with a zwitterionic polymer. *Nat. Biomed. Eng.* **2**, 894–906 (2018).
This article highlights the utility of antifibrotic coatings, improving the resilience of implanted biosensors.
82. Chou, Y.-N. et al. Ultra-low fouling and high antibody loading zwitterionic hydrogel coatings for sensing and detection in complex media. *Acta Biomater.* **40**, 31–37 (2016).
83. Kelley, E. L., Haridas, H. & Lorenz, C. Safety of microdose dexamethasone acetate in the eversense long-term implantable continuous glucose monitoring system. *Diabetes* **67**, 955 (2018).
84. Desai, T. & Shea, L. D. Advances in islet encapsulation technologies. *Nat. Rev. Drug Discov.* **16**, 338–350 (2017).
85. Schukur, L., Geering, B., Charpin-El Hamri, G. & Fussenegger, M. Implantable synthetic cytokine converter cells with AND-gate logic treat experimental psoriasis. *Sci. Transl. Med.* **7**, 318ra201 (2015).
86. Colton, C. K. Oxygen supply to encapsulated therapeutic cells. *Adv. Drug Deliv. Rev.* **67–68**, 93–110 (2014).
87. O'Connor, C., Brady, E., Zheng, Y., Moore, E. & Stevens, K. R. Engineering the multiscale complexity of vascular networks. *Nat. Rev. Mater.* **7**, 702–716 (2022).
88. Taylor, C. T. & Scholz, C. C. The effect of HIF on metabolism and immunity. *Nat. Rev. Nephrol.* **18**, 573–587 (2022).
89. Krock, B. L., Skuli, N. & Simon, M. C. Hypoxia-induced angiogenesis. *Genes Cancer* **2**, 1117–1133 (2011).
90. Yung, C. W., Barbari, T. A. & Bentley, W. E. Counteracting apoptosis and necrosis with hypoxia responsive expression of Bcl-2Delta. *Metab. Eng.* **8**, 483–490 (2006).
91. Ludwig, B. et al. Transplantation of human islets without immunosuppression. *Proc. Natl Acad. Sci. USA* **110**, 19054–19058 (2013).
92. An, D. et al. An atmosphere-breathing refillable biphasic device for cell replacement therapy. *Adv. Mater.* **31**, 1905135 (2019).
93. Coronel, M. M., Liang, J.-P., Li, Y. & Stabler, C. L. Oxygen generating biomaterial improves the function and efficacy of beta cells within a macroencapsulation device. *Biomaterials* **210**, 1–11 (2019).
94. Wang, L.-H. et al. An inverse-breathing encapsulation system for cell delivery. *Sci. Adv.* **7**, eabd5835 (2021).
95. Ochoa, M. et al. Integrated sensing and delivery of oxygen for next-generation smart wound dressings. *Microsyst. Nanoeng.* **6**, 46 (2020).
96. Toley, B. J. et al. Micrometer-scale oxygen delivery rearranges cells and prevents necrosis in tumor tissue in vitro. *Biotechnol. Prog.* **28**, 515–525 (2012).
97. Wu, H. et al. In situ electrochemical oxygen generation with an immunoisolation device. *Ann. N. Y. Acad. Sci.* **875**, 105–125 (1999).
98. Maharbiz, M. M., Holtz, W. J., Sharifzadeh, S., Keasling, J. D. & Howe, R. T. A microfabricated electrochemical oxygen generator for high-density cell culture arrays. *J. Microelectromechanical Syst.* **12**, 590–599 (2003).
99. Maleki, T. et al. An ultrasonically powered implantable micro-oxygen generator (IMOG). *IEEE Trans. Biomed. Eng.* **58**, 3104–3111 (2011).
100. Lee, I. et al. Electrocatalytic on-site oxygenation for transplanted cell-based-therapies. *Nat. Commun.* **14**, 7019 (2023).
101. Krishnan, S. R. et al. A wireless, battery-free device enables oxygen generation and immune protection of therapeutic xenotransplants in vivo. *Proc. Natl Acad. Sci. USA* **120**, e2311707120 (2023).
102. Shabestary, K. et al. Design of microbial catalysts for two-stage processes. *Nat. Rev. Bioeng.* https://doi.org/10.1038/s44222-024-00225-x (2024).
103. Bashor, C. J., Hilton, I. B., Bandukwala, H., Smith, D. M. & Veiseh, O. Engineering the next generation of cell-based therapeutics. *Nat. Rev. Drug Discov.* **21**, 655–675 (2022).
104. Cubillos-Ruiz, A. et al. Engineering living therapeutics with synthetic biology. *Nat. Rev. Drug. Discov.* **20**, 941–960 (2021).
105. U.S. Food and Drug Administration. Approved Cellular and Gene Therapy Products. *FDA* https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products (2024).
106. Ricotti, L. et al. Biohybrid actuators for robotics: a review of devices actuated by living cells. *Sci. Robot.* **2**, eaaq0495 (2017).
107. Nawroth, J. C. et al. A tissue-engineered jellyfish with biomimetic propulsion. *Nat. Biotechnol.* **30**, 792–797 (2012).
108. Cvetkovic, C. et al. Three-dimensionally printed biological machines powered by skeletal muscle. *Proc. Natl Acad. Sci. USA* **111**, 10125–10130 (2014).
109. Lee, K. Y. et al. An autonomously swimming biohybrid fish designed with human cardiac biophysics. *Science* **375**, 639–647 (2022).
110. Kim, Y. et al. Remote control of muscle-driven miniature robots with battery-free wireless optoelectronics. *Sci. Robot.* **8**, eadd1053 (2023).
This work leverages wireless optoelectronics to enable remote control of optogenetic muscle-powered biohybrid robots.
111. Tetsuka, H., Pirrami, L., Wang, T., Demarchi, D. & Shin, S. R. Wirelessly powered 3D printed hierarchical biohybrid robots with multiscale mechanical properties. *Adv. Funct. Mater.* **32**, 2202674 (2022).
112. Lynch, N. et al. Enhancing and decoding the performance of muscle actuators with flexures. *Adv. Intell. Syst.* **6**, 2300834 (2024).
113. Zhao, H. et al. Compliant 3D frameworks instrumented with strain sensors for characterization of millimeter-scale engineered muscle tissues. *Proc. Natl Acad. Sci. USA* **118**, e2100077118 (2021).
114. Dou, W. et al. Ultrathin and flexible bioelectronic arrays for functional measurement of iPSC-cardiomyocytes under cardiotropic drug administration and controlled microenvironments. *Nano Lett.* **23**, 2321–2331 (2023).
115. Rousseau, E. et al. Actuated tissue engineered muscle grafts restore functional mobility after volumetric muscle loss. *Biomaterials* **302**, 122317 (2023).
116. Pedersen, B. K. & Febbraio, M. A. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nat. Rev. Endocrinol.* **8**, 457–465 (2012).
117. Srinivasan, S. S., Maimon, B. E., Diaz, M., Song, H. & Herr, H. M. Closed-loop functional optogenetic stimulation. *Nat. Commun.* **9**, 5303 (2018).
118. Kim, Y. et al. Integration of graphene electrodes with 3D skeletal muscle tissue models. *Adv. Healthc. Mater.* **9**, 1901137 (2020).
119. Guo, X. et al. Tissue engineering the mechanosensory circuit of the stretch reflex arc with human stem cells: sensory neuron innervation of intrafusal muscle fibers. *Biomaterials* **122**, 179–187 (2017).
120. Cvetkovic, C., Rich, M. H., Raman, R., Kong, H. & Bashir, R. A 3D-printed platform for modular neuromuscular motor units. *Microsyst. Nanoeng.* **3**, 17015 (2017).
121. Kolar, K., Knobloch, C., Stork, H., Žnidarič, M. & Weber, W. OptoBase: a web platform for molecular optogenetics. *ACS Synth. Biol.* **7**, 1825–1828 (2018).
122. Ye, H., Daoud-El Baba, M., Peng, R.-W. & Fussenegger, M. A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. *Science* **332**, 1565–1568 (2011).
123. Shao, J. et al. Smartphone-controlled optogenetically engineered cells enable semiautomatic glucose homeostasis in diabetic mice. *Sci. Transl. Med.* **9**, eaal2298 (2017).
124. Folcher, M. et al. Mind-controlled transgene expression by a wireless-powered optogenetic designer cell implant. *Nat. Commun.* **5**, 5392 (2014).
125. Yu, Y. et al. Optogenetic-controlled immunotherapeutic designer cells for post-surgical cancer immunotherapy. *Nat. Commun.* **13**, 6357 (2022).
126. Müller, K., Engesser, R., Timmer, J., Zurbriggen, M. D. & Weber, W. Orthogonal optogenetic triple-gene control in Mammalian cells. *ACS Synth. Biol.* **3**, 796–801 (2014).
127. Zhang, Y. et al. Upconversion optogenetic engineered bacteria system for time-resolved imaging diagnosis and light-controlled cancer therapy. *ACS Appl. Mater. Interfaces* **14**, 46351–46361 (2022).
128. Krawczyk, K. et al. Electrogenetic cellular insulin release for real-time glycemic control in type 1 diabetic mice. *Science* **368**, 993–1001 (2020).
This work is an early example of a wireless biohybrid 'cell factory' deployed in vivo as a regulated therapy.
129. Huang, J., Xue, S., Buchmann, P., Teixeira, A. P. & Fussenegger, M. An electrogenetic interface to program mammalian gene expression by direct current. *Nat. Metab.* **5**, 1395–1407 (2023).
130. VanArsdale, E. et al. Electrogenetic signaling and information propagation for controlling microbial consortia via programmed lysis. *Biotechnol. Bioeng.* **120**, 1366–1381 (2023).
131. Pan, Y. et al. Mechanogenetics for the remote and noninvasive control of cancer immunotherapy. *Proc. Natl Acad. Sci. USA* **115**, 992–997 (2018).
132. Stanley, S. A., Sauer, J., Kane, R. S., Dordick, J. S. & Friedman, J. M. Remote regulation of glucose homeostasis in mice using genetically encoded nanoparticles. *Nat. Med.* **21**, 92–98 (2015).
133. Levskaya, A. et al. Synthetic biology: engineering *Escherichia coli* to see light. *Nature* **438**, 441–442 (2005).
134. Tabor, J. J. et al. A synthetic genetic edge detection program. *Cell* **137**, 1272–1281 (2009).
135. Zhu, L., McNamara, H. M. & Toettcher, J. E. Light-switchable transcription factors obtained by direct screening in mammalian cells. *Nat. Commun.* **14**, 3185 (2023).
136. Meyer, K., Lammers, N. C., Bugaj, L. J., Garcia, H. G. & Weiner, O. D. Optogenetic control of YAP reveals a dynamic communication code for stem cell fate and proliferation. *Nat. Commun.* **14**, 6929 (2023).
137. Abedi, M. H., Lee, J., Piraner, D. I. & Shapiro, M. G. Thermal control of engineered T-cells. *ACS Synth. Biol.* **9**, 1941–1950 (2020).
138. Stirling, F. et al. Synthetic cassettes for pH-mediated sensing, counting, and containment. *Cell Rep.* **30**, 3139–3148.e4 (2020).
139. Sloas, D. C., Tran, J. C., Marzilli, A. M. & Ngo, J. T. Tension-tuned receptors for synthetic mechanotransduction and intercellular force detection. *Nat. Biotechnol.* **41**, 1287–1295 (2023).
140. MacKay, J. L. & Kumar, S. Simultaneous and independent tuning of RhoA and Rac1 activity with orthogonally inducible promoters. *Integr. Biol. Quant. Biosci. Nano Macro* **6**, 885–894 (2014).
141. Li, H.-S. et al. Multidimensional control of therapeutic human cell function with synthetic gene circuits. *Science* **378**, 1227–1234 (2022).
142. Donahue, P. S. et al. The COMET toolkit for composing customizable genetic programs in mammalian cells. *Nat. Commun.* **11**, 779 (2020).
143. Weinberg, B. H. et al. High-performance chemical- and light-inducible recombinases in mammalian cells and mice. *Nat. Commun.* **10**, 4845 (2019).

144. Schwarz, K. A., Daringer, N. M., Dolberg, T. B. & Leonard, J. N. Rewiring human cellular input-output using modular extracellular sensors. *Nat. Chem. Biol.* **13**, 202–209 (2017).
145. Farahani, P. E. et al. pYtags enable spatiotemporal measurements of receptor tyrosine kinase signaling in living cells. *eLife* **12**, e82863 (2023).
146. Krawczyk, K., Scheller, L., Kim, H. & Fussenegger, M. Rewiring of endogenous signaling pathways to genomic targets for therapeutic cell reprogramming. *Nat. Commun.* **11**, 608 (2020).
147. Morsut, L. et al. Engineering customized cell sensing and response behaviors using synthetic notch receptors. *Cell* **164**, 780–791 (2016).
148. Toda, S., Blauch, L. R., Tang, S. K. Y., Morsut, L. & Lim, W. A. Programming self-organizing multicellular structures with synthetic cell-cell signaling. *Science* **361**, 156–162 (2018).
149. Wauford, N. et al. Synthetic symmetry breaking and programmable multicellular structure formation. *Cell Syst.* **14**, 806–818.e5 (2023).
150. Wang, N. B., Beitz, A. M. & Galloway, K. E. Engineering cell fate: applying synthetic biology to cellular reprogramming. *Curr. Opin. Syst. Biol.* **24**, 18–31 (2020).
151. Shakiba, N., Jones, R. D., Weiss, R. & Del Vecchio, D. Context-aware synthetic biology by controller design: engineering the mammalian cell. *Cell Syst.* **12**, 561–592 (2021).
152. Mimee, M. et al. An ingestible bacterial-electronic system to monitor gastrointestinal health. *Science* **360**, 915–918 (2018).
This article demonstrates an ingestible, fully wireless bacterial-based biohybrid sensor.
153. Servinsky, M. D. et al. Directed assembly of a bacterial quorum. *ISME J.* **10**, 158–169 (2016).
154. Mansouri, M. et al. Smartphone-flashlight-mediated remote control of rapid insulin secretion restores glucose homeostasis in experimental type-1 diabetes. *Small Weinh. Bergstr. Ger.* **17**, e2101939 (2021).
155. Aghlara-Fotovat, S. et al. Hydrogel-encapsulation to enhance bacterial diagnosis of colon inflammation. *Biomaterials* **301**, 122246 (2023).
156. Inda-Webb, M. E. et al. Sub-1.4 cm3 capsule for detecting labile inflammatory biomarkers in situ. *Nature* **620**, 386–392 (2023).
157. Zeh, N., Bräuer, M., Raab, N., Handrick, R. & Otte, K. Exploring synthetic biology for the development of a sensor cell line for automated bioprocess control. *Sci. Rep.* **12**, 2268 (2022).
158. Wei, X. et al. Efficacy and cardiotoxicity integrated assessment of anticancer drugs by a dual functional cell-based biosensor. *Sens. Actuators B Chem.* **283**, 881–889 (2019).
159. Roy, R., Ray, S., Chowdhury, A. & Anand, R. Tunable multiplexed whole-cell biosensors as environmental diagnostics for ppb-level detection of aromatic pollutants. *ACS Sens.* **6**, 1933–1939 (2021).
160. Graham, G. et al. Genome-scale transcriptional dynamics and environmental biosensing. *Proc. Natl Acad. Sci. USA* **117**, 3301–3306 (2020).
161. Wang, W. & Shao, Z. An intracellular sensing and signal transduction system that regulates the metabolism of polycyclic aromatic hydrocarbons in bacteria. *mSystems* **6**, e0063621 (2021).
162. Aydin, O. et al. Principles for the design of multicellular engineered living systems. *APL Bioeng.* **6**, 010903 (2022).
163. Mansouri, M., Ray, P. G., Franko, N., Xue, S. & Fussenegger, M. Design of programmable post-translational switch control platform for on-demand protein secretion in mammalian cells. *Nucleic Acids Res.* **51**, e1 (2023).
164. Praznik, A. et al. Regulation of protein secretion through chemical regulation of endoplasmic reticulum retention signal cleavage. *Nat. Commun.* **13**, 1323 (2022).
165. Vlahos, A. E. et al. Protease-controlled secretion and display of intercellular signals. *Nat. Commun.* **13**, 912 (2022).
166. Vlahos, A. E., Call, C. C., Kadaba, S. E., Guo, S. & Gao, X. J. Compact programmable control of protein secretion in mammalian cells. Preprint at *BioRxiv* https://doi.org/10.1101/2023.10.04.560774 (2023).
167. Beitz, A. M., Oakes, C. G. & Galloway, K. E. Synthetic gene circuits as tools for drug discovery. *Trends Biotechnol.* **40**, 210–225 (2022).
168. Purnick, P. E. M. & Weiss, R. The second wave of synthetic biology: from modules to systems. *Nat. Rev. Mol. Cell Biol.* **10**, 410–422 (2009).
169. Chen, Y. Y., Galloway, K. E. & Smolke, C. D. Synthetic biology: advancing biological frontiers by building synthetic systems. *Genome Biol.* **13**, 240 (2012).
170. Andrianantoandro, E., Basu, S., Karig, D. K. & Weiss, R. Synthetic biology: new engineering rules for an emerging discipline. *Mol. Syst. Biol.* **2**, 2006.0028 (2006).
171. Gao, X. J., Chong, L. S., Kim, M. S. & Elowitz, M. B. Programmable protein circuits in living cells. *Science* **361**, 1252–1258 (2018).
172. Bashor, C. J., Helman, N. C., Yan, S. & Lim, W. A. Using engineered scaffold interactions to reshape MAP kinase pathway signaling dynamics. *Science* **319**, 1539–1543 (2008).
173. Galloway, K. E., Franco, E. & Smolke, C. D. Dynamically reshaping signaling networks to program cell fate via genetic controllers. *Science* **341**, 1235005 (2013).
174. Lindner, F. & Diepold, A. Optogenetics in bacteria — applications and opportunities. *FEMS Microbiol. Rev.* **46**, fuab055 (2022).
175. Bird, L. J. et al. Engineering wired life: synthetic biology for electroactive bacteria. *ACS Synth. Biol.* **10**, 2808–2823 (2021).
176. Pekarsky, A. & Spadiut, O. Intrinsically magnetic cells: a review on their natural occurrence and synthetic generation. *Front. Bioeng. Biotechnol.* **8**, 573183 (2020).
177. Tanniche, I. & Behkam, B. Engineered live bacteria as disease detection and diagnosis tools. *J. Biol. Eng.* **17**, 65 (2023).
178. Chang, H.-J. et al. Programmable receptors enable bacterial biosensors to detect pathological biomarkers in clinical samples. *Nat. Commun.* **12**, 5216 (2021).
179. Moraskie, M. et al. Microbial whole-cell biosensors: current applications, challenges, and future perspectives. *Biosens. Bioelectron.* **191**, 113359 (2021).
180. Ravindran, P. T., McFann, S., Thornton, R. H. & Toettcher, J. E. A synthetic gene circuit for imaging-free detection of signaling pulses. *Cell Syst.* **13**, 131–142.e13 (2022).
181. Shui, S., Scheller, L. & Correia, B. E. Protein-based bandpass filters for controlling cellular signaling with chemical inputs. *Nat. Chem. Biol.* **20**, 586–593 (2023).
182. Ma, Y. et al. Synthetic mammalian signaling circuits for robust cell population control. *Cell* **185**, 967–979.e12 (2022).
Biohybrid devices will require synthetic population control systems, which this article demonstrates with paradoxical control.
183. Johnstone, C. P. & Galloway, K. E. Supercoiling-mediated feedback rapidly couples and tunes transcription. *Cell Rep.* **41**, 111492 (2022).
184. Frei, T. et al. Characterization and mitigation of gene expression burden in mammalian cells. *Nat. Commun.* **11**, 4641 (2020).
185. Jones, R. D. et al. An endoribonuclease-based feedforward controller for decoupling resource-limited genetic modules in mammalian cells. *Nat. Commun.* **11**, 5690 (2020).
186. Qin, C. et al. Precise programming of multigene expression stoichiometry in mammalian cells by a modular and programmable transcriptional system. *Nat. Commun.* **14**, 1500 (2023).
187. Cabrera, A. et al. The sound of silence: transgene silencing in mammalian cell engineering. *Cell Syst.* **13**, 950–973 (2022).
188. Blanch-Asensio, A. et al. STRAIGHT-IN enables high-throughput targeting of large DNA payloads in human pluripotent stem cells. *Cell Rep. Methods* **2**, 100300 (2022).
189. Zhang, M. et al. SHIELD: a platform for high-throughput screening of barrier-type DNA elements in human cells. *Nat. Commun.* **14**, 5616 (2023).
190. Aznauryan, E. et al. Discovery and validation of human genomic safe harbor sites for gene and cell therapies. *Cell Rep. Methods* **2**, 100154 (2022).
191. Johnstone, C. P., Wang, N. B., Sevier, S. A. & Galloway, K. E. Understanding and engineering chromatin as a dynamical system across length and timescales. *Cell Syst.* **11**, 424–448 (2020).
192. O'Connell, R. W. et al. Ultra-high throughput mapping of genetic design space. Preprint at *BioRxiv* https://doi.org/10.1101/2023.03.16.532704 (2023).
Large libraries and screening for circuit functions can be made more powerful as library sizes reach the scale for machine learning-guided design as demonstrated with CLASSIC.
193. Palmiter, R. D., Chen, H. Y. & Brinster, R. L. Differential regulation of metallothionein-thymidine kinase fusion genes in transgenic mice and their offspring. *Cell* **29**, 701–710 (1982).
194. You, L., Cox, R. S., Weiss, R. & Arnold, F. H. Programmed population control by cell-cell communication and regulated killing. *Nature* **428**, 868–871 (2004).
195. Elowitz, M. B. & Leibler, S. A synthetic oscillatory network of transcriptional regulators. *Nature* **403**, 335–338 (2000).
196. Danino, T., Mondragón-Palomino, O., Tsimring, L. & Hasty, J. A synchronized quorum of genetic clocks. *Nature* **463**, 326–330 (2010).

Acknowledgements

J.R., J.T.R., C.S., T.C.-K. and O.V. acknowledge funding from the Defense Advanced Research Projects Agency (DARPA) under agreement number FA8650-21-1-7119 and AWD00001596 as well as the Advanced Research Project Agency for Health under award number AY1AX000003. R.R. is supported by U.S. Department of Defense (DoD) Army Research Office Early Career Program (W911NF-22-1-0126), the National Science Foundation CAREER Program (2238715), and the U.S. DoD Office of Naval Research Young Investigator Program (N00014-24-1-2060). T.C.-K. further acknowledges funding support from the National Institutes of Health under award R01HL161106-02 and from the Army Research Office under cooperative agreement number W911NF-23-2-0138. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the official policies, either expressed or implied, of the Army Research Office or the Government of the USA. The Government of the USA is authorized to reproduce and distribute reprints for government purposes notwithstanding any copyright notation herein. K.E.G. acknowledges support from the National Institute of General Medical Sciences of the National Institutes of Health under award number R35-GM143033, by the National Science Foundation under CAREER Program award number 2339986, and from the U.S. Army Research Office under cooperative agreement W911NF-19-2-0026 for the Institute for Collaborative Biotechnologies.

Author contributions

All authors contributed to the conception, writing and revision of this Review. J.R. and K.E.G. made the figures.

Competing interests

J.T.R. is a co-founder of and claims equity and compensation from Motif Neurotech. J.R., J.T.R., T.C.-K., C.S. and O.V. are inventors on patent applications related to biohybrid cell therapy device concepts or components thereof. R.R. is an inventor on issued patents and patent applications related to biohybrid machines. K.E.G. declares no competing interests.

Additional information

Peer review information *Nature Reviews Bioengineering* thanks Adan Cohen and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

[1]Department of Biomedical Engineering, Northwestern University, Evanston, IL, USA. [2]Department of Materials Science and Engineering, Northwestern University, Evanston, IL, USA. [3]Center for Synthetic Biology, Northwestern University, Evanston, IL, USA. [4]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA. [5]Department of Electrical and Computer Engineering, Rice University, Houston, TX, USA. [6]Department of Bioengineering, Rice University, Houston, TX, USA. [7]Biotech Launch Pad, Rice University, Houston, TX, USA. [8]Department of Materials Science and Engineering, Carnegie Mellon University, Pittsburgh, PA, USA. [9]Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA. [10]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA. [11]Institute for Synthetic Biology, Rice University, Houston, TX, USA.