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Materials design by synthetic biology

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Abstract | Synthetic biology applies genetic tools to engineer living cells and organisms analogous to the programming of machines. In materials synthetic biology, engineering principles from synthetic biology and materials science are integrated to redesign living systems as dynamic and responsive materials with emerging and programmable functionalities. In this Review, we discuss synthetic-biology tools, including genetic circuits, model organisms and design parameters, which can be applied for the construction of smart living materials. We investigate non-living and living self-organizing multifunctional materials, such as intracellular structures and engineered biofilms, and examine the design and applications of hybrid living materials, including living sensors, therapeutics and electronics, as well as energy-conversion materials and living building materials. Finally, we consider prospects and challenges of programmable living materials and identify potential future applications.

Biologically inspired engineering, also called biomimicry, takes its cues from the rich diversity of forms and functions found in nature, and is applied across scales and disciplines[1]. For example, functional materials can be created by recapitulating design principles derived from nacre, spider silk or gecko toes, using artificial building blocks[2,3]. Biomimetic approaches hold boundless potential for optimizing specific material functionalities, because synthetic building elements can outperform their natural analogues in terms of mechanical properties and are readily manufactured on a large scale. However, challenges remain for mimicking the responsiveness and adaptiveness of biological systems, because it requires often complicated, top-down manufacturing tools that need to be coordinated with separate sensing and actuation modules[4,5]. Living creatures harness the power of evolution to optimize multiple subsystems based on universal building blocks, including nucleic acids, proteins and polysaccharides. Therefore, insights into the meticulous architecture and function of cells, tissues and organisms can inform engineering solutions guided by biology (or mimicking biology)[6].

Synthetic biology aims to program biological systems to perform user-defined functions[7]. Instead of computer codes, nucleic acid or protein sequences are used as scripts to direct the behaviour of biological systems from the subcellular to the organism level. Engineering principles, such as modular design, standardizing of parts and computational simulation, have fuelled the rapid advancement of synthetic biology, and, with the invention of the genetic toggle switch[8] and repressilator[9] in 2000, synthetic biology has emerged as a full-fledged engineering field. Engineering principles can be adopted for biological systems to transform cells into designed living machines; for example, ON–OFF state changes and oscillating protein concentrations can be engineered in bacteria, such as *Escherichia coli*. The same principle has facilitated the development of quantitative techniques to probe biological problems[9]. Concepts such as control theory[10] and elements such as logic gates[11] and modular parts[12] have been implemented in developing genetic circuits with predictable behaviours, substantially expanding the programmability of biological phenomena (FIG. 1). After two decades of intensive tool development, massive genetic circuits can now be built that perform sophisticated decision-making processes involving multiple inputs and outputs[13].

Complex biological functions created with model circuitry can further be modified with artificial functionalities. Advances in bioinformatics and the decreasing cost of DNA sequencing and synthesis have given rise to de novo biological systems that integrate sensing, computing and recording to perform specific tasks[14–16]. The applications of these technologies range from biomedicine[14] to agriculture[17].

Synthetic biology has also extended its impact to materials science and engineering (FIG. 1). Engineered biomaterials have great potential in a wide range of areas, including medicine[18], civil and environmental engineering[19], architecture[20] and product design[21]. Living organisms continually interact with their surrounding environment through the biomaterials they produce[22]. The properties of natural biomaterials are related to their biological function; for example, as living organisms grow and move, they generate extracellular matrices, cell walls and other biopolymers that serve as templates for

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composite formation tailored to fit specific physiological functions[23]. In these dynamic processes, the spatial and temporal information required for the production of biomaterials is encoded in the genome. Therefore, the synthesis and performance of biomaterials can be directed by designing genetic circuits to tune gene expression and biomolecular interactions with exquisite spatio-temporal control[22]. Indeed, synthetic biology can be applied to generate geometrical patterns[24] and to introduce new functionalities into model living-materials systems, such as *E. coli* biofilms[25]. Insights into gene-regulation mechanisms in more complex organisms have further allowed the design of self-organizing multicellular structures using synthetic cell–cell signalling, for example, for the asymmetric differentiation of mammalian cells[26].

Traditionally, genetic engineering has been used to create modified or fusion proteins that can be purified and processed into protein-based materials[27]. Similarly, metabolic engineering has allowed the synthesis of chemicals that can serve as monomers for the downstream production of polymeric materials[28]. However, although these materials are engineered in cells, they do not fully exploit the features of living biological systems[27–29]. Thus, to better capture the emphasis on the dynamics of living systems, we propose to denote the concept of designing materials with synthetic biology 'materials synthetic biology'. In materials synthetic biology, living systems are used to produce dynamic and responsive materials for user-defined applications. These materials can be endowed with new functions using programmable features, such as self-regeneration, remodelling in response to environmental cues and evolution[22].

In materials synthetic biology, designer cells and genetic circuitries are employed to engineer functional materials. The use of smart, programmable biomolecular or cellular devices cannot only improve our ability to replicate and harness the properties of natural materials but also improve artificial materials by incorporating biologically derived or inspired functionalities[23]. Therefore, synthetic biology holds great promise for materials design; however, this area remains underexplored because, historically, synthetic biology has focused on biomedicine. Furthermore, the concept of genetic circuits has only recently been introduced in materials science, which has mainly applied genetic engineering thus far. Materials synthetic biology also sheds light on mechanisms of biomaterial formation, which are difficult to decipher using traditional reverse-engineering approaches.

In this Review, we discuss the integration of synthetic biology and materials-science tools for the development of self-organizing functional materials and hybrid living materials. We highlight their specific advantages and challenges, and investigate how materials synthetic biology can exploit non-model biological systems for the design of new materials and applications. We also discuss how active biomolecular or living cellular components can improve the performance of artificial materials and how they can be used to build living hybrid composites with programmable functionalities. Finally, we examine the strengths and limitations of materials synthetic biology that need to be overcome to move towards real-world applications.

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Synthetic biology for materials design

Computational tools in combination with gene sequencing, synthesis and editing technologies enable the precise engineering of biomolecular and cellular functions. From the simple toggle switch to automated genetic-circuit design, synthetic-biology tools and design concepts for prokaryotic and eukaryotic systems have become increasingly sophisticated[7,30] (FIG. 1). These genetic tools allow the rational intervention in cellular processes, including genome replication, transcription, translation and post-translational modifications[7]. Genetic circuits can be implemented for the production of chemicals and biopolymers (such as DNA or proteins), produced either constitutively or in response to environmental cues, which can then be used for the engineering of materials. For the production of chemicals, a cellular metabolic network can be modified to redistribute the fluxes or to create new pathways for metabolite synthesis[31]. Modular engineering of biomolecular domains enables the engineering of distinct functionalities and hierarchical assemblies of biopolymers, for example, proteins with non-canonical amino acids[32], self-assembling DNA[33] and protein complexes[34]. The fine-tuning of the dynamic features of biomolecules using genetic circuits endows living systems with computer-like capabilities, including sensing, computing, recording and other programmable functions[35–37]. Programmable protein and nucleic-acid materials can also be designed and produced by in vitro purification and post-processing; although equally important, these materials do not require genetic circuits and, thus, they are not the focus of this Review.

Genetic circuits

Genetic circuits, which essentially perform computation inside a cell or in a cell-free reaction mix, can operate at the transcriptional, post-transcriptional, translational or post-translational level[7]. In each case, the inputs are the presence (or absence) of various environmental cues, whereas the outputs are the initiation (or inhibition) of RNA synthesis, protein synthesis or amino-acid-residue functionalization. Transcriptional circuits are the most

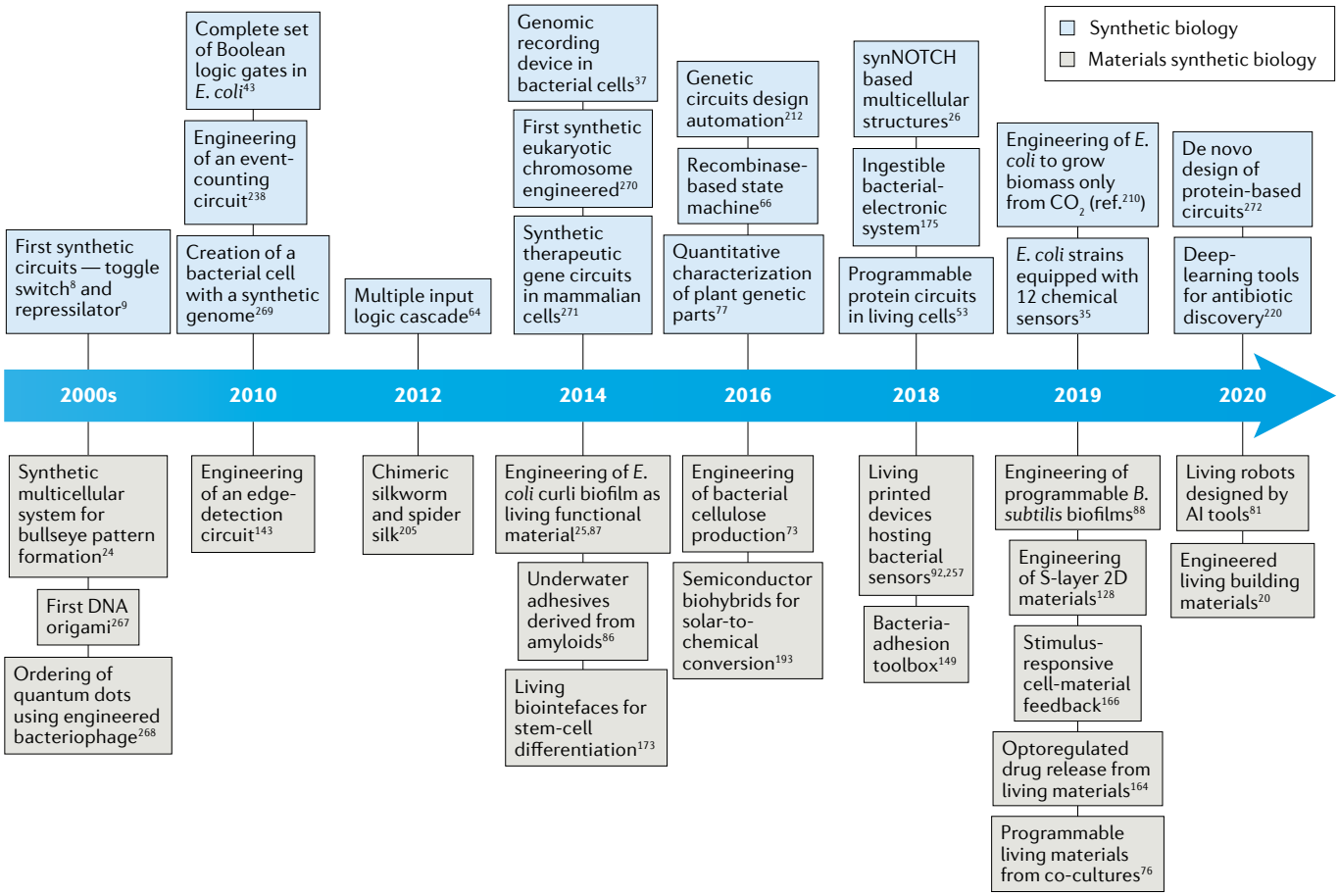
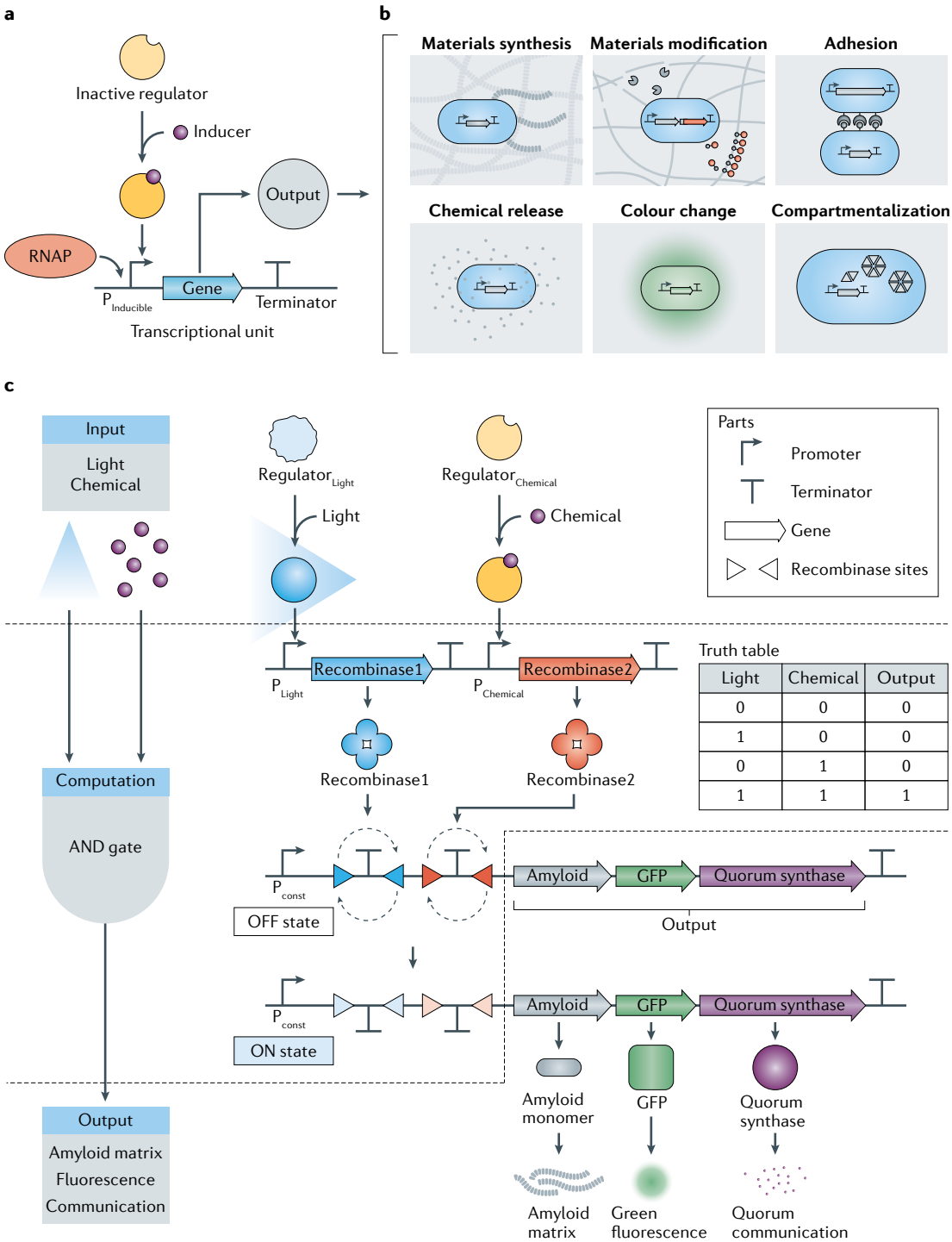


Fig. 1 | **Timeline of major milestones in synthetic biology and materials synthetic biology.** AI, artificial intelligence; *B. subtilis*, *Bacillus subtilis*; *E. coli*, *Escherichia coli*.

commonly used tools to manipulate gene expression by controlling the efficiency of RNA synthesis. The binding of a transcription factor (regulator) to a specific DNA sequence (operator) upstream of the gene of interest can either recruit RNA polymerase (RNAP) to initiate transcription or block RNAP attachment and, thus, prevent gene expression[7] (FIG. 2a). Depending on its mode of action, a transcriptional regulator can be either an activator or a repressor. In an inducible system, activation or deactivation by the regulator is determined by a conformational change or oligomerization triggered by certain inputs, such as exposure to light[38], temperature change[39] or binding to a chemical[40]. The DNA sequence that contains the operators with affinity for the regulators is called inducible promoter, which controls downstream gene expression based on environmental cues[35]. Alternatively, constitutive promoters can independently drive gene expression at a fixed level, which depends on the strength of the promoter[41]. A basic genetic circuit, or a transcriptional unit, is constructed by combining a regulator, the corresponding promoter, a gene of interest and a terminator sequence that ends transcription[42]. In materials synthetic biology, the genes of interest often encode proteins that influence the microscopic or macroscale properties of the material (FIG. 2b). Multiple transcriptional units can be linked or layered by connecting the output of the upstream transcriptional unit to the input of the downstream transcriptional unit; the resulting complex circuits can execute Boolean logic computation[43], amplify or integrate signals[44] or introduce delays in a cascade of processes[45].

The functionality of transcriptional circuits can be expanded using additional genetic tools. For example, memory can be introduced into the circuit design by incorporating recombinases. Recombinases excise or invert DNA fragments flanked by specific recognition sites[46], and, thus, they can be used to manipulate the presence and direction of promoters, genes of interest and terminators. For example, the excision or inversion of the coding sequence of a particular gene can completely shut down its expression[47]. Similarly, removing a terminator placed between a promoter and a gene of interest lifts the inhibition imposed by the terminator, turning the circuit from the OFF state to the ON state (FIG. 2c). Recombinases are useful for building switches and memory circuits, because they introduce permanent changes in the circuit topology, enabling digital control of gene-expression states[48] and ON–OFF switching of material production upon induction[49]. Powerful tools based on the CRISPR–Cas system can also be used for the tuning of the transcription status[50]. With the help of a guide RNA targeting a specific DNA sequence, an inactivated Cas9 protein can precisely bind to a promoter and interfere with RNAP function (that is, CRISPR



interference (CRISPRi)) or, if fused with an activator, help to recruit extra RNAP (that is, CRISPR activation (CRISPRa))[51]. In contrast to the 'digital switch' nature of recombinases, CRISPR–Cas-derived tools offer a more analogue 'tuning knob' to knock down or ramp up gene expression[13]. Tuning is potentially useful for the fabrication of materials with gradient features, for example, materials that undergo a gradual change in properties, such as stiffness or colouration.

Most materials designed by synthetic biology have been based on transcriptional circuits thus far, which are easy to implement, well characterized and versatile[52]. However, circuits operating at the translational and post-translational levels[53,54], such as RNA-based circuits involving miRNAs and toehold switches, are also being explored for the design of materials for theranostics[55,56]. Alternatively, protein-based circuits using phosphorylation, functional fusion peptides and polymerization provide prompt output responses to inputs, because they skip the rate-limiting protein-translation step[53]. Therefore, materials synthesized by a combination of multiple circuit types could accomplish highly sophisticated tasks, for example, fast and ultra-sensitive detection of a massive array of inputs while simultaneously performing

◀ Fig. 2 | **Genetic circuits. a** | The basic architecture of a simple inducible circuit. A transcriptional unit is a DNA fragment that has a promoter, a gene of interest and a terminator. An inducer, such as a small molecule, binds to a transcriptional regulator and activates it to recruit RNA polymerase (RNAP) to the promoter and start gene expression, which generates an output. **b** | Outputs of genetic circuits can be functional proteins relevant for the design of materials. **c** | An example of an AND gate that computes based on two inputs and generates an output consisting of three proteins. At the input level, two transcriptional regulators can sense two orthogonal inputs, light and a chemical, and control the expression of two different recombinases. When both inputs are present, Recombinase1 and Recombinase2 bind to their corresponding recognition sites on the output circuit and invert the terminators. The output proteins are only ON and produced when both terminators upstream of the genes are inverted, removing the obstacles preventing the RNAP from starting transcription. GFP, green fluorescent protein.

computation, data storage and even mechanical actuation. Such materials would outperform responsive materials made purely of artificial components.

Genetic parts

The choice of the regulator–promoter pair determines the sensing capability of materials equipped with transcriptional genetic circuits. Inducible transcriptional units can respond to natural chemicals, light, temperature change, and electrical and mechanical stimuli (TABLE 1), and have been optimized to be modular in both prokaryotic[7] and eukaryotic systems[30]. Alternatively, sensing modules can be generated de novo to create new inputs, such as artificial chemicals. De novo generation requires extensive genome mining or haphazard mutagenesis, and, thus, remains an immense challenge. However, protein-directed evolution techniques have shown great promise in expanding the current repertoire of inducible regulators[57].

The output of a genetic circuit determines the physical and chemical properties of a material, resulting in distinct functionalities (FIG. 2b, TABLE 1). In genetic circuits with sensing capabilities, the response of the circuit can be detected by a change in colour or opacity. For example, fluorescent or chromatic proteins, pigments or enzymes that generate bioluminescence can be expressed upon exposure to environmental inputs, without adding significant biomass to materials[58–61]. Hybrid materials with artificial and natural features can be created by combining biological sensors with abiotic materials[62]. To generate biological materials, naturally occurring polymers can directly serve as outputs of synthetic circuits. However, technical difficulties in recreating the native microenvironment for the in situ assembly of biological materials, for example, for spider-silk-fibre spinning[63], have limited the toolbox to simpler systems thus far, such as metabolites, carbohydrate polymers, structural protein monomers, enzymes and amyloid fibres (TABLE 1).

To achieve higher complexity in the material architecture, biochemical processes would have to be coordinated by genetic circuits. At the base level, digital computation based on ON and OFF states is commonly used for the detection of a specific cue from the environment or from an upstream cellular process. By linking multiple transcriptional units, simple Boolean operators, such as AND, OR and NOT gates, can be constructed using transcriptional regulators[64], recombinases[48] and CRISPR-related tools[65]. Using these fundamental parts, universal logic gates, such as NAND and NOR, can be constructed, enabling the development of logic gates with multiple inputs[43]. The computational result of logic gates either leads to an immediate output or can be registered on a recombinase-based state machine, for which the combination and order of inputs determine a specific state of the system[66]. The states and the controllable transition between states have laid the foundation for building multi-material systems, in which different elements can be produced in a specific order defined by the input sequence. Furthermore, recombinase-based state machines can be designed to mimic the cell-differentiation process or the evolution of cell states[66], and, thus, could be used to direct the growth and morphogenesis of cell-based living materials.

Cell–cell communication is essential for layered-circuit design and pattern formation to allow applications in materials at the systems level. Molecules, such as metabolites, peptides and proteins, can serve as signals indicating cell-population density and physical proximity[67,68]. Therefore, quorum-sensing molecules, mating factors and cell-surface receptors, which facilitate signal transduction between cells (TABLE 1), can be repurposed as circuit outputs that are exported to the cell surface or the extracellular space, where they act on other cells as inputs initiating responses. For example, *N*-acyl homoserine lactone, the primary quorum-sensing molecule in Gram-negative bacteria, can amplify upstream signals[69] and create patterned biofilm materials[25].

Choice of organism

Using living systems as a chassis for materials design builds on native cellular machineries, which have evolved as self-replicating entities that respond to the environment[22]. However, for applications that require a high degree of homogeneity and predictivity, purified biomolecules and cell-free systems may be preferable[27,29]. The extent to which an organism can be engineered depends on the availability of its genomic sequence. Computational tools support the prediction and annotation of promoters, genes and terminators based on genome databases[70]. An ideal chassis is characterized by a thorough characterization of its genetic parts (native or foreign) and the availability of tools for genetic transformation, because developing new tools is laborious and time-consuming. Therefore, pioneering work on materials powered by synthetic biology has primarily focused on model microorganisms, such as *E. coli* and *Saccharomyces cerevisiae*. These organisms usually have rapid growth rates, and an extensive collection of genetic tools is already available, making them ideal organisms for prototyping genetic circuits and for expressing foreign biomolecules in a plug-and-play fashion. Genetic circuits use the resources of the host cell to perform tasks and, thus, compatibility of circuit parts with the host cellular machinery (transcription, translation or molecule secretion) needs to be individually optimized. Otherwise, unexpected resource competition, crosstalk and toxicity may lead to failures in material functions[13].

Biofilms produced by model microorganisms demonstrated great promise for incorporating new functionalities by genetic fusion of functional proteins (for example, enzymes) or through interfacing

Table 1 | **Genetic parts and applications for materials design**

Genetic part	Type	Examples	Refs
Input			
Chemical	Small molecules	IPTG (pLac-LacI); arabinose (pBad-AraC)	229
	Heavy-metal ions	Arsenic (pArs-ArsR); mercury (pMer-MerR)	90,154
	Biomolecules	Steroid (pLexA-XVE); haem (pHrt-HrtR)	175,230
Electrical	Redox potential change	pSox-SoxR	180
Optical	Red light	Cph8/OmpR; phyB/PIF	140
	Green light	CcaS/R	140
	Blue light	YF1/fixJ; Cry2/CIB1; EL222	140,231
Thermal	Heat	Heat-shock-response mechanism	39
	Cold	Cold-shock-response mechanism	232
Mechanical	Pressure	Mechanosensitive channels	233
Computation			
Boolean logic	AND gate	Riboregulators; recombinases; split regulators; regulator cascades; CRISPR–Cas	64,234
	NAND gate		235
	NOR gate		43,48,236
Memory	Recording	Retrons; self-targeting CRISPR–Cas	37,237
	Timer	Feedforward loop	40
	Counter	Recombinase cascade	238
State change	Toggle switch	Repressor feedback loops	8
	Oscillator	Repressor cascade	9,146
	State change	Recombinase-based state machine	66
Communication			
Diffusion (chemicals)	Quorum sensing	AHL (lux, rhl, las, cin, tra, rpa)	24,67
Diffusion (peptides)	GPCR-based sensing	Yeast mating factor	68
Contact	Surface receptor	synNotch	26
Output			
Fluorescence	Fluorescent proteins	GFP; RFP; BFP	58
Bioluminescence	Luciferases	Firefly luciferase; NanoLuc	59
Colour change	Chromoproteins	aeBlue; amilCP; tsPurple	60
	Pigments	Carotenoid; melanin	61,239
	Opacity change	Cephalopod reflectin	112
Bioplastics	Monomers for bioplastic	Polyhydroxyalkanoates	240
Electricity	Current production	Extracellular electron transfer	241
	Radical polymerization		131
Protein materials	Amyloid fibres	Curli (CsgA); TasA	25,87,88
	Adhesives	Mussel foot proteins	86,101
	Adhesins	Substrate-binding peptides; nanobodies	87,149,242
	Silk	Silkworm silk; spider silk	205,243
	Protein ligase	SpyTag-SpyCatcher	244
Polysaccharide materials	Cellulose	Bacterial cellulose	73,130
	Chitin and chitosan	GlcN and GlcNAc	245
Mineralization	Magnets	Ferritin; magnetosome organelle	246,247
	Calcium carbonate	Microbially induced $CaCO_3$ precipitation	248
	Silica	Diatom silaffin	102
	Quantum dots	CdSe; CdS	19,249
Acoustic property	Gas vesicle	Gas-vesicle-forming proteins	89,113

AHL, N-acyl homoserine lactone; BFP, blue fluorescent protein; CP, chromoprotein; GlcN, glucosamine; GlcNAc, N-acetylglucosamine; GPCR, G protein-coupled receptor; IPTG, isopropyl β-D-1-thiogalactopyranoside; GFP, green fluorescent protein; RFP, red fluorescent protein; synNotch, synthetic Notch.

with abiotic materials, such as inorganic nanoparticles in proof-of-concept work[23]; however, they often lack macroscale structural robustness and they need to be combined with artificial scaffolds. Thus, there has been a shift towards engineering unconventional organisms that natively produce large amounts of extracellular matrix (ECM); for example, acetic-acid bacteria, which naturally exhibit a high yield of ECM consisting of bacterial cellulose — a material with exceptional mechanical properties[71]. Species such as *Gluconacetobacter xylinus* and *Komagataeibacter rhaeticus* have also gained popularity for the development of genetic tools because they can be programmed by genetic circuits transplanted from *E. coli*[72,73]. Similarly, mycelium-producing fungi, such as *Ganoderma lucidum* and other mushrooms, which are used commercially as structural and packaging materials[74], can be genetically engineering using CRISPR-Cas9, which makes them an attractive platform for the production of responsive materials equipped with programmable gene circuits[75]. Alternatively, co-culture of a model organism with a materials-producing organism could provide a balance between engineerability and bulk biopolymer production, such as fermented food with a symbiotic community of yeast cells. For example, a synthetic kombucha pellicle can host engineered yeast and wild-type acetic-acid bacteria, forming co-cultures that function as biosensing cellulosic materials[76].

Engineering multicellular systems that include animal or plant cells is technically more challenging than building microbial systems. Slower growth rates and more stringent culture conditions make it more difficult to rapidly prototype eukaryotic cells[77,78]. However, animals and plants are relevant to real-world applications and, therefore, building biomaterials with their cellular components is a key focus of living functional materials research[26,79]. For example, genetic circuits allow animal cells to form 3D tissue-like structures made of multiple cell types, paving the path towards tunable autonomous organoids[80] and living robots[81]. Similarly, circuit-equipped plants can gain additional functionalities, such as desalination and detection of hazardous agents in the environment; these functionalities build upon the native ability of plants to produce bulk cellulose and lignin composites[82,83], as well as recalcitrant biopolymers, including suberin[84] and sporopollenin[85]. Complex circuits with powerful computation capabilities validated in simple microbial systems could realize their full potential in engineered animal and plant living materials in the future.

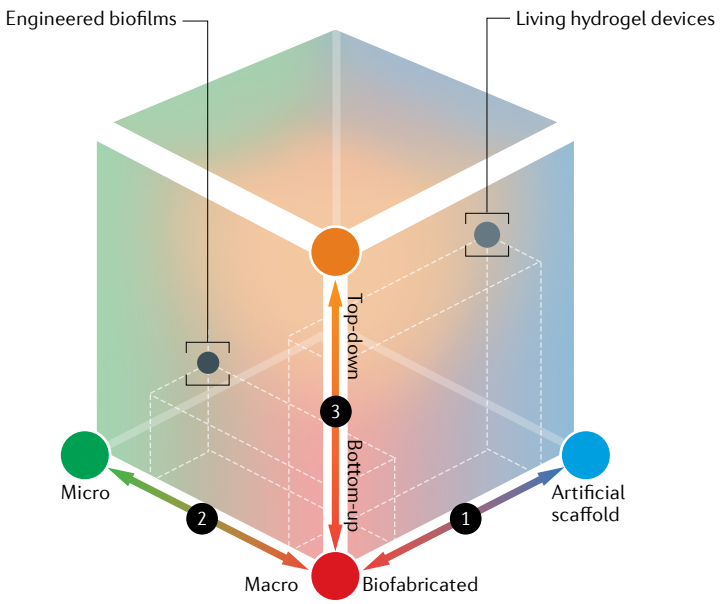


Fig. 3 | **Design parameter space for materials synthetic biology.** The cube represents the design space determined by three major parameter axes. (1) Following red to blue, the materials can be fabricated by biological processes carried out in living cells or they can be produced on abiotic artificial scaffolds. (2) Following green to red, materials are described based on their length scale, from microscopic to macroscopic. (3) Following red to orange, materials can be assembled by bottom-up approaches or top-down methods. Engineered biofilms produce their matrix using biopolymers from cells that are self-organized at the microscale. Hydrogel-based wearable devices contain engineered whole-cell biosensors, which are manufactured with predefined geometries at the macroscale.

Design parameter space

Self-organizing functional materials and hybrid living materials differ in their biomaterial composition. Self-organizing functional materials contain only biomolecules or living cells, whereas hybrid living materials also incorporate synthetic components, for example, artificial scaffolding matrices. Self-organizing functional materials include non-living and living materials, whose structural components are directly generated or derived from biological systems, rather than from artificial sources (FIG. 3). Functions generated by recombinant genes or genetic circuits can be harvested for the design of self-organizing multifunctional materials[52], for example, non-living materials, such as underwater protein adhesives recapitulating features of natural marine glues[86], and living materials, such as engineered living functional materials based on biofilms[87,88], bacterial cellulose[76] or intracellular assemblies[89], for diverse applications, such as bioremediation[90], biomedicine[18] and adhesion[91]. By contrast, hybrid living materials integrate living cells with non-biological components; for example, engineered cells can be combined with artificial matrices and biomanufacturing tools to form living devices with defined geometry and size[92,93]. In theory, such hybrid living materials can comprise components whose functions can combine and even synergize, which enables the integration of living attributes of cells with synthetic scaffolds and, thus, new sensing, recording and actuating capabilities not currently associated with synthetic materials.

The design parameter space is also related to the degree to which artificial scaffolds are used, the length scale at which they operate, ranging from microscale (biofilms)[87] to macroscale (building materials)[20], and to the design approach, for example, bottom-up morphogenesis[25] versus top-down design (casting and 3D printing)[92] (FIG. 3).

Self-organizing functional materials

Biological systems have self-assembly mechanisms that create functional materials across a broad spectrum of length scales using basic building blocks[94] (FIG. 4). At the nanoscale, the structure and function

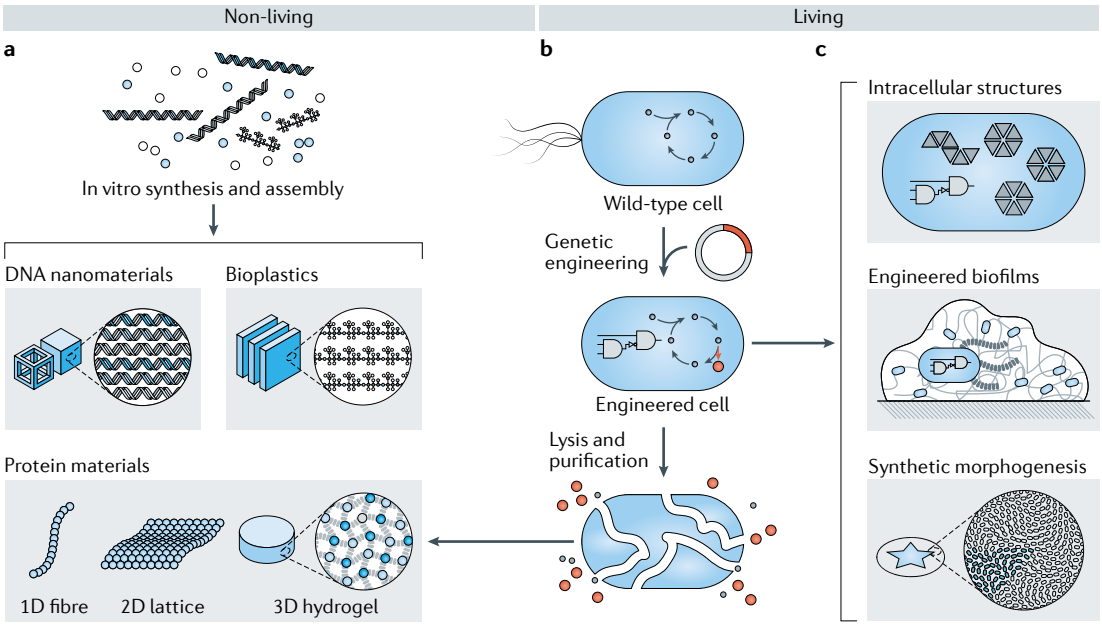


Fig. 4 | **Non-living and living self-organized materials. a** | Non-living DNA nanomaterials and bioplastics are usually artificially synthesized using chemicals or purified monomers from bioreactors. Protein materials, for example, 1D fibres, 2D lattices and 3D hydrogels, are mostly produced by processing purified proteins from genetically modified living cells. **b** | Cells undergo genetic engineering and acquire DNA-encoded information for producing precursors for bioplastics and monomers for protein materials. **c** | Engineered cells can be used as living materials with their functionalities programmed by genetic circuits that can create intracellular structures, improve the responsiveness of biofilms and direct synthetic-pattern formation and morphogenesis.

of biomacromolecules, such as nucleic acids and proteins, are determined by the sequential arrangement of nucleotides and amino acids, respectively. Similarly, at the population level, cells organize themselves into ordered architectures, based on genetic instructions encoded in their genome that orchestrate cell differentiation. Therefore, extracted or synthesized biopolymers (for example, nucleic acids, bioplastics and proteins) can be used for the fabrication of self-organizing, non-living, functional materials[27,28,95,96]. Importantly, synthetic-biology tools can be applied for the engineering of programmable living materials.

Non-living materials

Non-living functional materials can be made from self-organizing biomolecules (for example, nucleic acids, bioplastic precursors and proteins), derived from artificial synthesis or metabolic engineering[28,97] (FIG. 4a). For materials production, synthetic biology provides numerous natural or artificially designed modules with various functions, and enables their rational reassembly for customized applications[27,98,99]. For example, programmable CRISPR-responsive DNA hydrogels constructed by integrating CRISPR-associated nucleases with structural DNA elements, which can convert biological information (that is, guide RNA) into particular properties of materials (for example, conductivity or the ability to detect a virus)[100], or hierarchical strong underwater adhesives made from rationally designed recombinant proteins, composed of cohesive (self-assembling amyloids) and adhesive domains (3,4-dihydroxy-L-phenylalanine (DOPA))-containing mussel foot proteins)[86,101]. In addition, synthetic biology provides solutions for the in vivo production and functionalization of engineered materials in genetically modified organisms with remodelled metabolic pathways[28,95,102–105] (FIG. 4b), for example, genetically encoded DNA nanostructures[33], mechanics-tunable bioplastics[106] and recombinant proteins with modified functional moieties[107].

Living materials

In contrast to non-living materials, engineered living materials are composites of biopolymers and genetically modified cells (FIG. 4c). The living organisms hosting the genetic circuits in these materials can perform sensing, computation and actuation, allowing them to synthesize or modify the materials in response to environmental cues[23]. In addition to performing complex tasks, engineered living materials self-replicate and evolve, which makes them autonomous, adaptive and very versatile[22]; for example, the assembly of intracellular structures, the enhancement of biofilms by secreted materials or pattern formation by populations of cells.

Intracellular structures. Cells build intracellular structures for diverse purposes, including the formation of diffusion barriers or compartments for local confinement of biomolecules, enabling site-specific cellular functions[108]. Similar to organelles, these nanostructures or microstructures are constructed through the self-organization of biomolecules, such as proteins and lipids. Investigation of the self-assembly mechanisms of protein complexes, amyloids and viral capsids has

generated knowledge from which design principles for intracellular structures can be discerned[109], providing a blueprint for the reprogramming of protein materials using modular designs for the de novo creation of form–function relationships. For example, protein-based hydrogels[110] and phase-separated clusters[34] can be constructed in living cells by rationally designing the intermolecular interactions between peptide and protein modules. These droplet-like hydrogel materials, which provide an ideal microenvironment for biochemical reactions, are responsive to inputs, such as proteases and light[109]. In addition to influencing metabolism, intracellular protein assemblies, such as ferritin aggregates[111], reflectin-based structures[112] and gas vesicles[89,113], endow the cell with inducible materials properties, including magnetism, opacity and acoustics.

Engineered biofilms. Microbial communities often live in biofilms, which are composed of living cells embedded in a self-produced ECM. Secreted ECM polymers, such as proteins and polysaccharides, form 3D structures that protect cells against environmental challenges and provide a medium for nutrient exchange[114]. The underlying mechanisms of biofilm formation have been extensively studied because biofilm formation is intrinsic to many persistent, antimicrobial-resistant bacterial infections[114,115]. The increase in understanding of the protein and polysaccharide secretion machinery enables the repurposing of biofilms into assembly lines for functional materials production[23,52]. Synthetic-biology tools, such as genetic circuits, modular protein design and metabolic engineering, allow the creation of a broad spectrum of programmable functional biofilms.

Intracellular and extracellular amyloid fibres are formed by highly ordered protein aggregates[116]. In addition to their roles in the pathogenesis of neurodegenerative diseases, amyloids often act as functional protein structures in microorganisms[117]. For example, in enterobacteria, such as *E. coli* and *Salmonella* spp., curli amyloid fibres are the main component of the ECM, facilitating surface binding and promoting host colonization[118]. The CsgA protein monomer, which is the basic building block of *E. coli* curli fibres, served as one of the earliest chassis for biofilm engineering[87]. The production of curli fibres can be precisely tuned by putting the expression of CsgA under the control of inducible promoters, for example, promoters responding to small molecules[25]. Similarly, optical inputs can drive the on-demand production of curli fibres, allowing light-patterning of adhesive biofilms[119]. To further functionalize the biofilm, exogenous protein modules can be fused to the amyloid-forming domain of CsgA, leading to curli fibres capable of electrical conduction[120,121], enzymatic catalysis[122,123], bioremediation[90,124] and templating inorganic materials[125,126]. To enable more complex tasks, functionalized curli fibres can work in concert with computation and communication modules (TABLE 1), achieving simple Boolean decision-making[49] and intra-fibre patterning[25], or performing autonomous damage repair as 'smart' living glues[127]. In addition to the *E. coli* curli system, programmable TasA amyloid fibres in *Bacillus subtilis* can also be engineered into functional materials, demonstrating that modular design is a common feature of amyloid-forming proteins[91]. Engineered *B. subtilis* biofilms exhibit hydrogel-like viscoelastic behaviours, making them ideal for the manufacturing of protrusion-based additives[88]. Besides amyloids, surface-layer proteins, such as RsaA of *Caulobacter crescentus*, can be engineered using protein fusion to create functionalized, lattice-based 2D living materials[128].

Polysaccharides, including bacterial cellulose, are the main constituents of most mechanically strong biofilms[114]. The chemical and physical properties of polysaccharides can be altered by genetically modifying the pathways synthesizing the constituent monosaccharide building blocks[129]. However, this approach requires detailed knowledge of the polymerization-secretion machinery and well-developed genetic-engineering techniques, which are often lacking in non-model, ECM-rich microorganisms. The development of genetic toolkits for *K. rhaeticus* has greatly improved the engineerability of cellulose-producing strains, making inducible bacterial-cellulose production possible[73,130]. Alternatively, co-culture systems consisting of a potent polysaccharide-producing species and an engineerable model species bypass the hurdle of producing secreted polysaccharides[76]. For example, a kombucha-inspired living material has been engineered using *K. rhaeticus*, which generates a bacterial cellulose matrix, and *S. cerevisiae*, which provides programmable functionalities, such as light sensing and catalytic activity[76].

Living materials are currently mainly engineered using endogenous biomacromolecules for ECM synthesis; however, non-biological monomers can also be applied for building polymers in the extracellular space[131–133]. *Shewanella oneidensis*, an electroactive bacterium with a built-in extracellular electron-transfer machinery, allows metabolically controlled atom-transfer radical polymerization inside living cells using non-biological monomers and metal catalysts[132]. Such technologies, coupled with genetic circuits, could substantially broaden the biochemical spectrum for biofilm engineering.

Synthetic morphogenesis. Biological materials are rarely formed by a single cell type or a homogeneous population of cells. Living organisms self-organize into various spatial patterns that translate into heterogeneity of materials properties. By analysing natural biomaterials, macroscopic forms and functions can be mapped to distinctive cell types on the microscale. The ability to develop patterns and translate them into materials properties has enabled organisms to optimize their biomaterials, which is difficult to translate to artificial biomimetic products. The process of materials production in living cells is guided by spatially and temporally controlled functions determined by DNA-encoded information, which eventually leads to cell differentiation and morphogenesis[134–136]. For example, hierarchically self-organized functional cellular assemblies differentiating from the same progenitor cells can form skin tissue or insect exoskeletons, both of which have desirable mechanical properties that have emerged

from precisely combining and self-organizing different cell types. Technologies to precisely manipulate microscale components in synthetic materials are still in development. Alternatively, engineered living cellular factories can be equipped with coordinated synthetic genetic circuits to create autonomously self-organized materials[52,137]. Ideally, a seed cell carrying a genetic blueprint could replicate and differentiate into synthetic multicellular systems to perform preprogrammed functions while adapting to the surrounding environment, without external human intervention or guidance[138].

Programming morphogenesis has long been the Holy Grail for synthetic biologists[139]. Constructing genetic circuitry for the bottom-up orchestration of a series of biological events is extremely difficult, owing to the inevitable error propagation and restricted parameter space in living systems. As a starting point, simple synthetic optical inputs can be used to direct pigment-based pattern formation in biofilms[38]. This strategy can be expanded to produce multicolour inputs[140] and functional outputs such as adhesion[141]. The same approach can also be applied for chemical inputs[73] and further improved by computer-aided design to generate complex patterns[21]. Coupled with quorum-sensing molecules, these simple inputs can trigger downstream cell–cell communication, leading to semi-autonomous patterns, such as bullseye[24], stripes[142] and edge detection[143]. Furthermore, inspired by the reaction-diffusion model[144], stochastic Turing patterns can be constructed in biofilms using quorum-sensing molecules with different diffusivities[145]. Tools such as repressilators[146] and synthetic asymmetric cell division[147,148] can also serve as orthogonal mechanisms that could be used in parallel to achieve greater design complexity.

Adhesion between cells plays a pivotal role in determining the final multicellular geometry of 3D structures. Modular cell–cell adhesion enabled by synthetic adhesins provides a tool to rationally define the morphology of bacterial microstructures[149]. In mammalian systems, programmed cell adhesion combined with the synthetic Notch (synNotch) receptors juxtacrine signalling platform results in self-organizing, multilayered structures capable of sequential assembly and differentiation[26]. Aided by computational tools powered by artificial intelligence, robust and autonomous living tissues or materials could soon become a reality.

The mechanisms underlying pattern formation in natural sophisticated materials or structures (for example, diatom frustules) are being increasingly deciphered, and artificial pattern-generating circuits are being invented. Thus, it may soon be possible to create artificial living materials recapitulating the hierarchically ordered architectures and the outstanding materials properties of their natural counterparts.

Hybrid living materials and devices

Hybrid living systems incorporate cellular 'factories' and abiotic environmental components, which form composites with new properties[23]. For example, porous silica structures encapsulating single diatom cells (diatom frustules) not only provide the organism with robust mechanical support but they also take part in cellular metabolic processes, such as chlorophyll synthesis[150]. Synthetic materials manufactured by energy-intensive, top-down processes may exhibit specific user-designed properties, but lack biological responsiveness and adaptability. For artificial materials to acquire specific biological properties, such as self-adjustment, self-regeneration, self-healing and environmental responsiveness, synthetic materials can be coupled with living systems. In turn, the incorporation of high-performance synthetic components improves the performance and mechanical integrity of living materials. Thus, hybrid living materials have the advantages of artificial components and living cells, broadening the application scope of conventional composites (FIG. 5a), including sensing, therapeutics, electronics, energy conversion and building materials (TABLE 2).

Living sensors

The efficient, innate sense-and-response mechanisms in living cells, coupled with genetic modularity engineered by synthetic biology, offer vast possibilities for the construction of whole-cell biosensors[151]. Compared with traditional physical or chemical sensing methods, the manipulation of biosensors does not require sophisticated lab instruments or professional personnel. In contrast to cell-free sensing systems[152], the living components, which colonize the surroundings, enable on-site signal readout. Living biosensors are currently applied in various areas, including monitoring of metabolic production[153], environmental hazards[154] and disease signals[14].

Hybrid living sensors can be built by integrating genetically encoded microorganisms with biocompatible scaffolding materials (TABLE 2). For example, dipsticks produced by vacuum-filtering G protein-coupled receptor (GPCR)-refactored *S. cerevisiae* onto cellulose filter paper enable visible colour readouts upon contact with specific fungal mating peptides. This living sensor provides a scalable and economical platform for the global surveillance of fungal pathogens[155] (FIG. 5b). Long-term detection can be achieved using biocompatible hydrogel materials infused with water and nutrients to provide semi-liquid environments that can sustain cell survival and the exchange of molecules[156]. Owing to their tunable viscoelastic properties, these materials can be processed with various fabrication tools, such as moulding and 3D printing, making them interesting scaffolds for field-deployable biosensors[92,157]. In addition to their cell-protective role in harsh environments, hydrogel materials can also help to reduce potential risks of environmental pollution from leaked genetically modified microorganisms. For example, a bilayer hydrogel comprising a robust, porous hydrogel shell and a bacteria-containing alginate core can serve as effective biocontainment to inhibit the escape of genetically encoded microbes[93].

However, living sensing materials also have several shortcomings, including limited detection sensitivities and operational ranges, which are determined by the cells[158]. These limitations can be addressed by optimizing of the sensing modules, for example, by modifying the strength of transcriptional promoters[159],

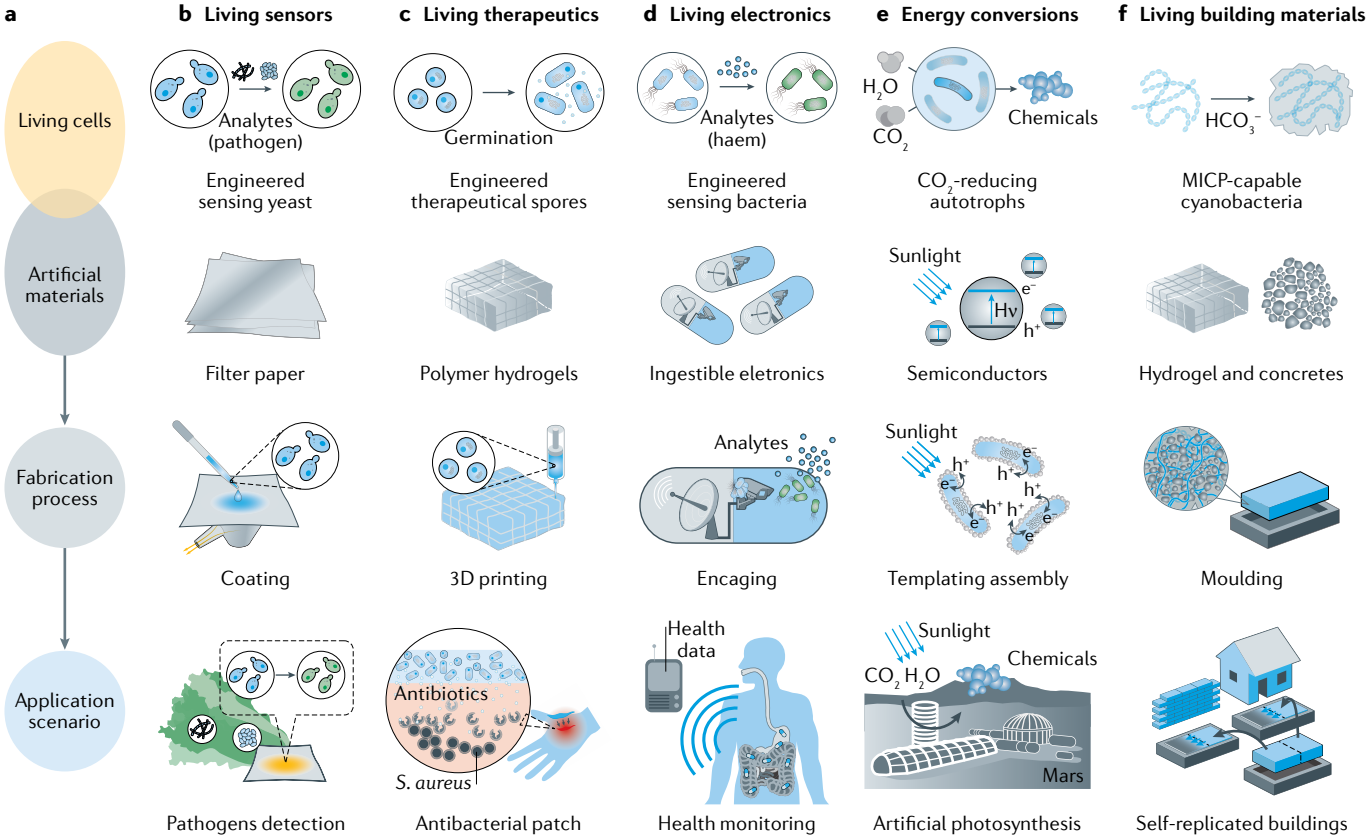


Fig. 5 | **Hybrid living materials. a** | Hybrid living materials are engineered by integrated living cells and artificial materials. **b** | Living paper dipsticks for detecting pathogens. Pathogen-detection yeasts coated on filter paper can be used to detect vegetable pollutants. **c** | Living therapeutic patches for blocking wound infection. The 3D-printed patch containing antibiotic-secreting *Bacillus subtilis* spores inhibits pathogen infection. **d** | Living electronics for monitoring gastrointestinal health. Blood-inducible microbial sensors in microelectronic devices can be used for diagnosing gastrointestinal diseases. **e** | Renewable energy production by artificial photosynthesis. Non-photosynthetic autotrophs interfaced with light-harvesting semiconductors can be used for chemical production in space. **f** | Self-replicated living bricks. Cyanobacteria with microbially induced calcium-carbonate precipitation (MICP) capabilities enable the biofabrication of living building bricks. S. *aureus*, *Staphylococcus aureus*.

adjusting translational levels[160] or controlling post-translational degradation[44]. Alternatively, signal-cascade genetic circuits[44] can be amplified and cellular consortia[161] can be constructed to achieve ultra-sensitive detection thresholds and tunable input and output operational ranges.

Living therapeutics

Native and genetically modified microorganisms have been traditionally employed in industry to produce bioactive metabolites. Viable cells equipped with drug-producing genetic modules constitute living therapeutics, engineered to prevent or treat diseases in vivo[162]. Living therapeutics can be administered for the sustained and long-term treatment of chronic diseases; however, to avoid immune responses (immunosuppression) associated with the uncontrolled cell growth of free-floating microorganisms in the body, the organisms have to be encapsulated within biocompatible scaffolding materials[163].

Similar to hybrid biosensors, biocompatible nutrient-containing hydrogels with mechanical robustness and selective penetrability are the preferable artificial materials for fabricating living therapeutic devices. For example, soft, biocompatible agarose hydrogels can support the survival of genetically modified *E. coli*, which secretes drugs into culture media in response to light. Owing to the optoregulation of metabolic pathways, the composite material can be dynamically tuned by light to regulate reporter production, localization and dose release[164,165]. Similarly, biopolymer-based microcapsules and nanoporous membranes can serve as cell containments, ensuring matter exchange between cells and surroundings, and providing continuous nutrient supply for trapped cells[166,167].

Hybrid living therapeutics can not only deliver drugs inside the body but they can also be applied for the treatment of pathogen infections on skin. Antibiotic-producing microbes entrapped in soft, hydrated hydrogels can be used as cost-effective medical bandages with long-term or on-demand antimicrobial properties. For example, a 3D-printed wound-shaped hydrogel patch that contains *B. subtilis* spores excreting lysostaphin and thiocillin can be applied to skin wounds to detect and kill *S. aureus*[168] (FIG. 5c). Genetically modified bacteria can also manipulate mammalian cell behaviours by secreting

Table 2 | **Hybrid living materials**

Material	Living components	Non-living components	Fabrication process	Refs
Living sensors				
Chemicals-detecting tattoo; patches	Rham; DAPG; IPTG; aTc; AHL-sensing *Escherichia coli*	Pluronic F-127-DA; polyacrylamide; alginate gels	Casting and moulding; 3D printing	92,157
Heavy-metal-ion-detecting beads	Cadmium-sensing *E. coli*	Polyacrylamide; alginate gels	Casting and moulding	93
Biomolecule-detecting gels	L-histidine-sensing *Saccharomyces cerevisiae*	Polyacrylamide gels	Casting and moulding	250
Fungal-pathogen-detecting dipsticks	Fungal-mating-peptides-sensing yeast	Cellulose filter papers	Direct deposition	155
Buried-landmine-detecting beads	DNT-sensing or TNT-sensing *E. coli*	Alginate hydrogel	Casting and moulding	156
Living therapeutics				
Skin transplants	Cellulose-secreting *Acetobacter xylinum*	Hyaluronic acid; κ-carrageenan; fumed silica	3D printing	251
Implantable or ingestible devices	Native or engineered human cells (for example, HEK293T cells, cardiac stromal cells and insulin-secreting β cells)	Encapsulation materials (for example, polydimethylsiloxane-based macrodevice, poly(vinyl alcohol) microneedle patches and alginate hydrogels or capsules)	Encaging; casting and moulding	163,252–254
Antibacterial devices	Drug-secreting and antibiotic-secreting microorganisms (spores, *E. coli*, *Bacillus subtilis*, *Penicillium chrysogenum*, lactobacilli)	Pluronic F-127; hydrogels (agar, agarose, alginate, dextran); microcapsule (alginate-based); porous polycarbonate membrane	3D printing; casting and moulding; encaging; layer-by-layer manufacturing	165,167,168,174,255
Stem-cell differentiation	*Lactococcus lactis*	Glass and poly(ethyl acrylate) surfaces	Biofilm adhesion	172,173
Living electronics				
Gut-health-monitoring pills	Blood-sensing *E. coli*	Wireless microelectronics	Encaging	175
Implantable mesh electrical probes	Neurons	Nanowire field-effect-transistor detectors	Neuron cells adhered to the detectors	256
Diabetes-treating electrogenetic interface	Electrosensitive insulin-secreting human β cells	Microelectronic implants	Encaging	181
Resettable pressure sensors	Programmable biofilm-secreting *E. coli*	Gold nanoparticles	Templating assembly	257
Energy conversions				
Microbial fuel cell (chemical to electricity)	Electrochemically active organisms (for example, *Shewanella oneidensis* MR-1; *Geobacter sulfurreducens*)	Conductive polymers (for example, PEDOT); carbon-based materials (for example, graphene oxide); polyelectrolyte (for example, CPE-K); tailored metal electrodes (for example, 3D porous electrodes)	Direct deposition	186,258,259
Biophotovoltaics (solar energy to electricity)	Cyanobacterium or microalgae	Conductive materials (for example, graphene nanoribbons, PEDOT:PSS polymers); complementary light-harvesting materials (for example, ZnO nanorods)	Direct deposition	188,190
Artificial photosynthesis (solar to chemical)	Natural autotrophs (for example, *Moorella thermoacetica* and *Sporomusa ovata*); genetically engineered microbes (for example, *E. coli* and *S. cerevisiae*)	Light-harvesting nanostructures (gold clusters, semiconductors); light-capturing electrochemical devices	Templating assembly; direct deposition	125,193–195,260–262
Living building materials				
Biofabricated bricks	Calcium-carbonate-precipitation-capable bacteria (for example, cyanobacterium)	Sand; soil	Casting and moulding	20
Biodegradable foams	Mycelium (for example, *Ganoderma lucidum*)	Agricultural waste	Casting and moulding	263
Self-healing concretes	Calcium-carbonate-precipitation-capable bacteria (for example, *B. pseudofirmus*)	Mortar concretes	Casting and moulding	198

Table 2 (cont.) | **Hybrid living materials**

Material	Living components	Non-living components	Fabrication process	Refs
Others				
Biofouling-resistant membranes	NO-producing and H_2O_2-producing *E. coli*	Nanofiltration membranes	Direct deposition	264
Bioremediation devices	Phenol-degrading *Pseudomonas putida*	Hyaluronic acid	3D printing	251
Biomanufacturing platform	Metabolite-producing *E. coli* or *S. cerevisiae*	Hydrogels or chitosan capsules	Casting and moulding; encaging	166,265,266
Self-cleaning surface	*P. roqueforti*	Agar gels and porous polycarbonate membrane	Layer-by-layer manufacturing	167
Wearable biohybrid devices	Fluorescence-producing *E. coli*	Agar/agarose hydrogels	3D printing	21,267

AHL, N-acyl homoserine lactone; aTc, anhydrotetracycline; CPE-K, conjugated polyelectrolyte-K; DAPG, 2,4-diacetylphloroglucinol; DNT, 2,4-dinitrotoluene; PEDOT:PSS, poly(3,4-ethylenedioxythiophene) polystyrene sulfonate; Pluronic F-127-DA, Pluronic F-127 diacrylate; Rham, rhamnose; TNT, 2,4,6-trinitrotoluene.

metabolites[169] and, thus, they could also be used in regenerative medicine. For example, the non-pathogenic bacterium *Lactococcus lactis*, which can be engineered to display recombinant human fibronectin ($FNIII_{7–10}$)[170] on extracellular biofilms, colonizes organic or inorganic surfaces and forms 'living biointerfaces', which support the differentiation of human mesenchymal stem cells[171–173].

Synthetic biology offers possibilities to engineer living systems with custom-built functions by rewiring genetic circuits[7]. Therefore, drug-releasing cells could also be developed for other healthcare applications, such as low-cost cosmetics or face masks with virolytic capability. In addition to the dynamic features of engineered living systems, artificial synthetic materials can also endow hybrid composites with customized properties (for example, responsiveness). For example, a smart, adaptable gel made of thermo-responsive polymer Pluronic F-127 that contains living *B. subtilis* spores can be applied to treat superficial fungal infections. This gel converts from the liquid to the hydrogel state when the temperature rises to 37 °C (REF.[174]).

Living electronics

Programmable cells can be integrated with electrical devices to simplify detection processes of biosensors and to enable remote and real-time control of living materials; for example, an ingestible micro-bio-electronic device composed of encapsulated bacteria and electronic photodetectors[175] can monitor gastrointestinal health. Upon sensing a particular biomarker, for example, haem, N-acyl homoserine lactone or thiosulfate, the bacteria within the device produce luminescence, which is detected by a photodetector that wirelessly transmits photocurrent data in vivo to an external device for real-time monitoring. This device, although still at an early stage of development, may be beneficial for diagnosing and monitoring otherwise difficult-to-detect health conditions (FIG. 5d). In addition to photodetector-embedded microelectronics, the integration of electrochemical electrodes[176], field-effect-transistor devices[177] or genetically engineered electrode-reduction microbes[178] also enable environmental monitoring and health diagnostics by converting cellular biochemical changes to easily detectable electrical signals.

Electronic devices can also remotely control the behaviour of engineered living materials. For example, a 'HydrogeLED' implant connects the digital signal of a far-red light-emitting diode (LED) to optogenetically responsive cells. The implanted device can release drugs in vivo for the treatment of diabetes and can be remotely controlled by smartphones[179]. In this device, cell behaviour is directly linked to electrical stimulation. Electron-triggered gene expression has also been explored for an *E. coli* SoxR-mediated transcription system in an electrogenetic device[180]. However, the bacteria require anaerobic culturing environments and the system may be too toxic for in vivo applications[180]. External digital electronic inputs can modulate mammalian cells that contain depolarization-based genetic circuits[181]. Electrogenetic interfaces can be constructed by coupling electrosensitive insulin-secreting β cells with a wireless electrical device, enabling electro-triggered insulin delivery in vivo[181]. These examples of living bioelectronics demonstrate the potential of materials synthetic biology for real-time sensing applications.

Energy-conversion materials

Hybrid living devices are being explored for the generation of renewable energy, providing an important contribution to mitigating the global energy and environmental crisis. For example, microbial fuel cells, which rely on viable exoelectrogens, can convert energy from organic matter into electrical power[182]. Exoelectrogens, such as the model bacteria *S. oneidensis* or *Geobacter* spp., transport electrons via redox proteins attached to the outer membrane or pili nanowires and via indirect redox electron shuttles[183]. The efficiency of electron transfer from the cytoplasm to the external electrode is crucial for the performance of microbial fuel cells. Synthetic biology can be applied to improve electron generation from exoelectrogens and optimize their conductive pathways[184]. Artificial materials, including 3D porous bioaffinity anodes[185] and conductive coating materials (such as reduced graphene oxide or polypyrrole), can be used to form artificial biofilms, which further improve electron delivery[186].

Biological photovoltaics derived from microbial fuel cells use photosynthetic microorganisms, such as microalgae or cyanobacteria, to harvest and convert

solar energy into electricity. However, in this type of energy-conversion device, the efficiency of transferring photoexcited electrons to electrodes remains limited[187]. Similar to microbial fuel cells, hybridization of conductive substrates and addition of biocompatible soluble mediators can improve the electron transfer between cells and electrodes[188]. Considering that only 45% of the solar spectrum (visible light) can be absorbed by photosynthetic cells[189], complementing living cells with additional light-capturing materials (such as plasmonic hybrid nanostructures, for example, ZnO nanorods/Au nanoparticles[190]) can help harvest solar energy from broad wavelength ranges. The intracellular and extracellular electron transportation can also be improved by introducing electron-exporting conduits from exoelectrogens into photosynthetic cells using synthetic biology; however, the metabolic bottleneck of haem-containing proteins remains a challenge for the engineering of cyanobacteria[191].

Artificial photosynthetic systems, which consist of native autotrophic microbes and semiconducting materials or external light-harvesting devices, enable highly selective solar-to-chemical energy conversion[192]. For example, photoexcited electrons from light-absorbing semiconductor nanoparticles can be used by the non-photosynthetic acetogen *Moorella thermoacetica* to create reducing equivalents, accelerating the CO_2 fixation process[193] (FIG. 5e). Synthetic biology can be applied to introduce engineered solar-to-chemical metabolic pathways into model microorganisms. For example, *E. coli* encoding hydrogenase can be loaded onto light-capturing materials to catalyse the production of H_2 in anaerobic illuminated environments[194]. Electrons can also be photogenerated by yeast-bound inorganic semiconductors for the regeneration of the redox cofactor nicotinamide adenine dinucleotide phosphate (NADPH), enabling the efficient synthesis of high-value-added metabolites[195]. These living energy-conversion materials are typically built by coupling engineered strains with non-living semiconductor components. To improve their performance, the components and cells need to be integrated through an interface, which will require a better fundamental understanding of electron transfer between the components.

Living building materials

Living systems can also be applied for building construction. For example, inspired by the phenomenon of microbially induced calcium-carbonate precipitation, bacterial bricks were invented by directly culturing calcium-carbonate-precipitating bacteria with mortar in brick moulds[196]. Biomineralization directly occurs in the mixture and promotes the aggregation of separate inorganic particles, leading to the formation of bricks with high mechanical strengths. This process avoids traditional clay-heating procedures and massive carbon emissions, and the final products are eco-friendly and able to self-replicate if placed under benign conditions (appropriate temperature and humidity)[20] (FIG. 5f). Building materials that contain viable mineral-precipitating microorganisms also exhibit self-healing properties[197]. If the concrete surface is damaged, dormant bacteria exposed to cracks germinate upon contacting the outside air and moisture, which triggers specific metabolic activities, such as ureolysis, methane oxidation and photosynthesis. The metabolic changes lead to an increase in the precipitation of the surrounding calcium carbonate and, thus, enables damage repair[197]. Long-term survival of microbes is the most salient factor in determining the performance of these self-healing materials. Owing to the inhospitable conditions (dehydration, low oxygen and high pH) in concrete materials, calcium-carbonate-precipitating microbes have to tolerate high pH and heat, and, generally, they should have the ability to form spores. Biocompatible carriers, such as microcapsules and hydrogels, can function as protective shelters[198]. In addition, synthetic biology can be applied to introduce anti-desiccation components, for example, from tardigrades[199] or by stress-selective evolution[200], which improve the resilience of microbes. Moreover, biomineralization-relevant metabolic pathways could be modified or nucleating sites could be engineered on biofilms to improve the mechanical strength and to shorten the healing process.

Fast-growing mycelium can also be applied to construction. The divergent filaments of mycelia can function as self-organized ropes that can robustly bind substrate particles (for example, wood chips) to composite materials with advantageous properties (such as compostable, light weight, fire resistant and soundproof)[201]. In mycelium-based materials, the filaments spread autonomously and rapidly form an integrated material. This approach could be particularly useful in low-resource areas (for example, to build airport runways in wartime or temporary shelters in deserts). Only a few genetically modified mycelium materials have been explored thus far; however, advances in fungal genome editing[75], in inhibiting mushroom fruiting bodies[202] and in developing synthetic sense-to-response circuits[35] will contribute to the creation of living buildings with user-defined functions, such as the release of fragrance or absorption of external toxic or greenhouse gases. Mycelium-based materials are also currently limited by low compressive strength and low stiffness[203]. To overcome these limitations, inspiration can be taken from plants, which possess outstanding mechanical strength and toughness, owing to the oriented arrangement of polysaccharide cellulose and the formation of lignin–carbohydrate complexes[204]. The incorporation of structural order and molecular interactions by synthetic biology may provide a viable route to improving the mechanical performance of fungi-based materials.

Outlook

Synthetic biology has facilitated the development of a new class of smart materials for biomedical, environmental and consumer applications. These smart materials display a wide range of length scales, design approaches and matrix types. However, limitations remain owing to inherent problems related to biological engineering (FIG. 6).

Currently, mainly model organisms, such as *E. coli*, are used as chassis for materials production or as the active component in composites. However, model

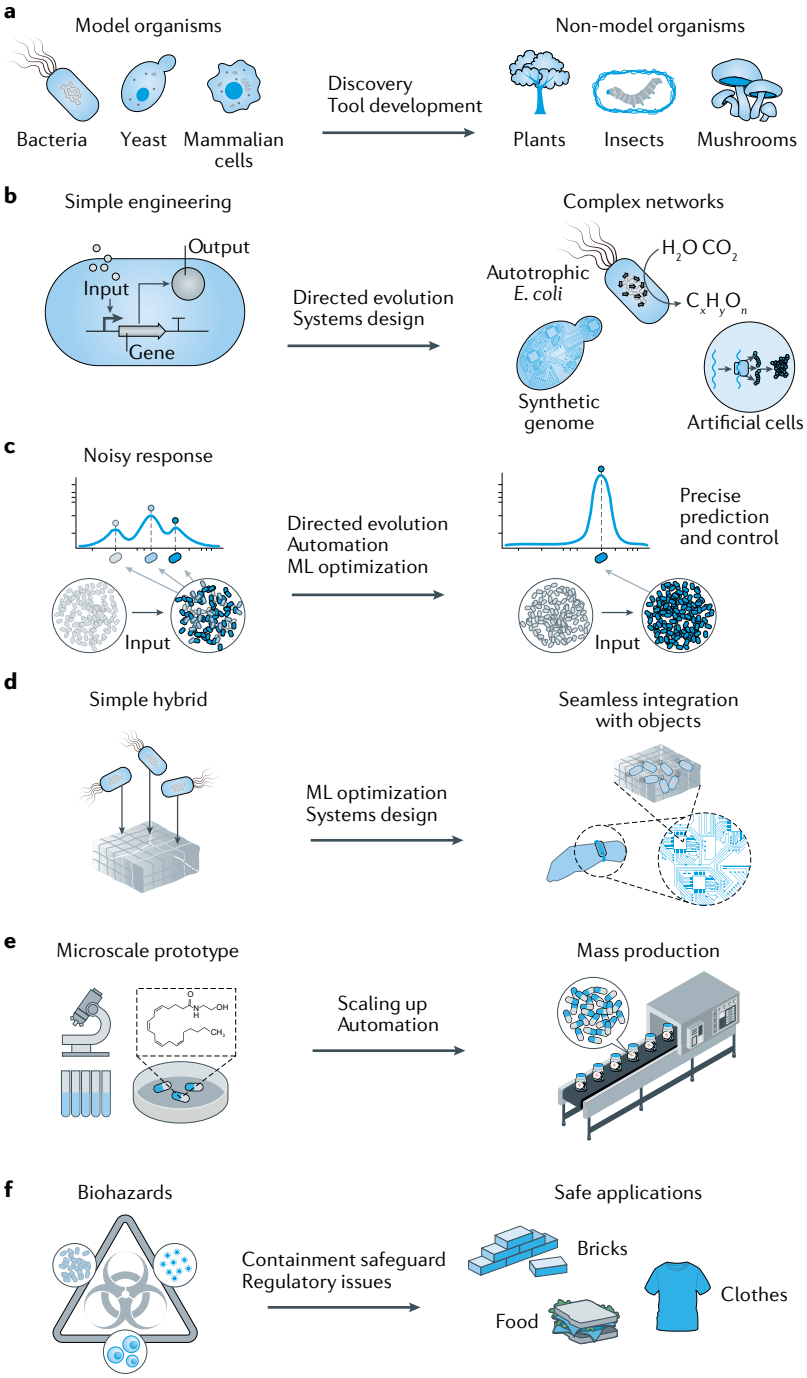


Fig. 6 | **Challenges and future directions of materials synthetic biology. a** | Research in materials synthetic biology is currently limited to well-characterized model systems that were chosen because of high engineerability. As more genetic tools are discovered and developed, unconventional organisms, which are potent material producers, are predicted to become the major organisms in the field. **b** | Using directed evolution and systems design, large-scale and complex circuitry can be computationally generated and replace simple designs with few transcriptional units. **c** | Biological input–output functions are often noisy and error-prone. Optimization using machine learning (ML) and automation at design and test levels will greatly improve the precision of cellular responses and benefit computational simulations. **d** | Instead of a simple mixture of artificial materials and engineered cells, future living devices will seamlessly integrate biotic and abiotic parts that work in concert to perform complex tasks. **e** | To bring an early-stage prototype to the market, manufacturing processes need to be redesigned with a focus on scalability and automation, which are often lacking in a laboratory set-up. **f** | Biocontainment safeguards and relevant regulations need to be implemented to ensure safe application of materials in the real world. *E. coli*, *Escherichia coli*.

organisms are often chosen because they are easy to engineer, not because they are competent material producers. Genetic-engineering tools can also be applied to modify non-model organisms; however, they depend heavily on reliable genome sequences and efficient transformation and screening methods, whose development is often time-consuming and labour-intensive. Advances in sequencing and genome-editing technologies will enable the engineering of non-model organisms, for example, silkworms[205], mushrooms[75] and vascular plants[77], with great potential for robust biopolymer generation (FIG. 6a). Organisms exhibiting complicated dynamic multicellular behaviours, such as slime moulds[206], are also potential candidates for active material development. In addition, mining newly sequenced genomes is likely to lead to the discovery of genetic parts with new properties, such as regulator–promoter pairs for the sensing of chemicals that are currently not detectable by biosensors[207].

New functionalities may also be generated by modifying genetic parts using directed evolution. By iterating mutagenesis followed by careful screening, for example, for enzymatic activities, cells can be modified to metabolize non-conventional substrates and produce chemicals for biomaterials synthesis more efficiently[208,209] (FIG. 6b). For example, by employing metabolic rewiring coupled with directed evolution, engineered *E. coli* can use carbon dioxide as the only carbon source, which makes it autotrophic and, thus, ideal for sustainable biomaterials production[210]. Similar selective concepts could also be applied to attributes such as adhesiveness and stiffness, which will require tailored optimization for high-throughput screening. In addition to engineering proteins that directly contribute to materials properties, directed evolution can also be applied to optimize promoter–regulator pairs to reduce background activation, increase sensitivity and expand the dynamic range[35]. Such improvements would benefit the construction of computational models for genetic circuits, which require the precise quantification of input–output functions (FIG. 6c).

As predictive power has increased, the complexity and scale of genetic circuitry in model organisms have grown exponentially. Upscaling poses challenges at the circuitry level, because the assembly of layers of genetic units often results in failures with unknown causes. Integrating modules from various sources requires a tremendous amount of characterization, design and fine-tuning, which are often laborious processes if done manually. Thus, standardized genetic parts and syntaxes play crucial roles in creating a universal programming language that operates across platforms and species[70]. Using automated computer-aided genetic parts[211], circuit design[212] and modular DNA assembly[213], large and multilayer networks can be implemented to design materials that cannot be engineered with simple topologies and a limited number of transcriptional units; for example, synthetic genomes[214] and artificial cells[215] could be constructed. Generalizing the high-throughput characterization of individual parts, coupled with automation[212], is necessary at the in silico circuit-design level and at the testing stages, in particular, because biomass-generating outputs often create a substantial metabolic burden and

can lead to resource competition with other modules in the circuit architecture[216,217]. An automated workflow assisted by robotics[218] to characterize the responses of materials-related circuits would enable the production of a large amount of data for establishing quantitative models from data-driven computational tools.

The rapid growth of machine learning and artificial intelligence has also impacted materials engineering and synthetic biology[219,220]. The large training datasets generated by automated experimental platforms allow machine-learning techniques to predict biomolecular behaviours without the need to understand the underlying mechanisms[221]. In particular, protein materials engineering benefits from deep learning, which has enabled the rational design of structures and functions, despite limited knowledge of protein folding[222]. Similarly, machine learning could outperform current mechanistic models for the systems design of complex genetic networks. Beyond the cellular level, the collective behaviour of a population of cells and its emerging materials properties are difficult to predict, because cell populations are dynamic and influenced by the environment. This complexity is reflected in the gap between proof-of-concept hybrid materials, such as simple mixtures of cells and hydrogels, and mature products, which require the systematic amalgamation of living and non-living components, often on a much larger scale. In particular, cell growth, packaging and communication with the device, demand systems that take all relevant parameters into consideration (FIG. 6d). With the help of machine learning and artificial intelligence, we envision that the seamless integration of cells and objects could soon become a reality, and engineered cells interfacing with electronics could lead to products for medical and environmental applications.

For real-world applications, scalability and safety remain major concerns for materials powered by synthetic biology. Unicellular microorganisms, such as *E. coli*, *B. subtilis* and *S. cerevisiae*, which are the current focus of research in biopolymer-precursor production[29] and biofilm-based functional materials[52], are among the primary candidates entering the industry for biomaterials production. In chemical production, cell cultures grown in a small batch of test tubes exhibit drastically different behaviours compared with cell cultures grown in industrial bioreactors. Optimizing growth conditions, such as accessibility to gas and nutrient transport at high liquid volume, to enable maximal metabolic flux is greatly improved by high-throughput screening and automation with robotics[223]. However, industrial optimization has not yet been achieved for the mass production of engineered-biofilm-derived materials, which have only been demonstrated at the nanoscale and microscale thus far (FIG. 6e). Finally, safety issues are important hurdles preventing genetically modified organisms from entering the market. Chemical containment, for example, kill switches[224] and synthetic auxotrophy[225], can prohibit the propagation of engineered cells outside controlled environments. In addition, physical containment strategies using abiotic materials can prevent the escape of engineered cells[93]. Regulations must be carefully developed alongside technological advances, and impacts at the social, ethical, economic and environmental levels need to be considered[226–228] (FIG. 6f). A well-established regulatory system for materials synthetic biology could also facilitate the standardization of manufacturing procedures and outcomes.

Materials design by synthetic biology opens the possibility of creating a new class of materials with tailored morphologies and functions. The core of these materials is living cells or biomolecules that can perform sensing, computation and actuation. The interdisciplinary field of materials synthetic biology has tremendous potential for the sustainable fabrication of smart biomaterials.

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All authors contributed to the discussions, writing and reviewing of the article content. T.-C.T., B.A. and C.Z. prepared the figures and tables.

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