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Review article

# Engineering the next generation of theranostic biomaterials with synthetic biology

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ABSTRACT

Biomaterials have evolved from inert materials to responsive entities, playing a crucial role in disease diagnosis, treatment, and modeling. However, their advancement is hindered by limitations in chemical and mechanical approaches. Synthetic biology enabling the genetically reprograming of biological systems offers a new paradigm. It has achieved remarkable progresses in cell reprogramming, engineering designer cells for diverse applications. Synthetic biology also encompasses cell-free systems and rational design of biological molecules. This review focuses on the application of synthetic biology in theranostics, which boost rapid development of advanced biomaterials. We introduce key fundamental concepts of synthetic biology and highlight frontier applications thereof, aiming to explore the intersection of synthetic biology and biomaterials. This integration holds tremendous promise for advancing biomaterial engineering with programable complex functions.

## 1. Introduction

Biomaterials have evolved significantly since their inception, progressing from inert and static materials to interactive materials capable of responding to dynamic microenvironments within the human body [1]. Concurrently, advancements in medicine have expanded the scope of biomaterials applications beyond traditional use in tissue engineering and regenerative medicine. Biomaterials now play a crucial role in theranostics, enabling the diagnosis and treatment of a wide range of diseases, as well as the establishment of disease models. These emerging applications impose new demands on biomaterials, necessitating increased complexity, precision, specialized functions, and expedited research and development approaches. In recent years, many efforts have been made to improve the responsiveness and tunability of biomaterials [2–5]. However, the inherent limitations of chemical and mechanical approaches have significantly hindered the development of biomaterials with advanced functionalities, calling for a new paradigm to enhance material programmability.

Synthetic biology can program biological systems with user-defined functions leveraging forward-engineering principles and methodologies from molecular biology. This interdisciplinary field assembles biological systems at different levels, ranging from biological molecules to multicellular systems, and employs engineering principles of abstraction, standardization, and modularity. Over the past decades, synthetic biology has made significant achievements, particularly in the realm of cell programming (Fig. 1) [6–13]. Researchers excavating the potential of cells have successfully engineered designer cells capable of executing artificial functions, either by introducing minor genetic modifications or constructing synthetic gene circuits within the cells. Such cellular functions have been programmed to solve diverse global concerns, such as food production, fuel shortage, environment remediation, and medical management. In particular, diagnostic or therapeutic cells have been designed to report the presence of disease-related biomarkers or provide treatments like drug secretion and tumour eradication. Moreover, synthetic biology extends beyond programming cellular functions and encompasses the engineering of cell-free biological systems, such as the rational design of biological macromolecules (DNA, RNA, proteins) and the fabrication of de novo raw materials (unnatural amino acids, artificial genetic codes) (Table 1). Collectively, synthetic biology demonstrates tremendous potential in programming complex functions and holds promise for advancing biomaterials engineering [14].

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**Abbreviations**

| | |
|---|---|
| AD | Activation domain |
| ADSC | Adipose tissue-derived stem cell |
| AHL | Acyl-homoserine lactone |
| BMP-2 | Bone morphogenetic protein-2 |
| BMP-7 | Bone morphogenetic protein-7 |
| CAR | Chimeric antigen receptor |
| Cas | CRISPR-associated |
| Ccl2 | Chemokine (C–C) motif ligand 2 |
| CIB1 | Calcium and integrin-binding protein 1 |
| CRISPR | Clustered regularly interspaced short palindromic repeats |
| CRISPRa | CRISPR activation |
| CRY2 | Cryptochrome 2 |
| DBD | DNA-binding domain |
| dCas9 | Endonuclease deficient Cas9 |
| dECM | Decellularized extracellular matrix |
| DFU | Diabetic foot ulcer |
| Dox | Doxycycline |
| *E. coli* | *Escherichia coli* |
| ECM | Extracellular matrix |
| EcN | *Escherichia coli* Nissle 1917 |
| flaB | Flagellin B |
| GAG | Glycosaminoglycan |
| GI | Gastrointestinal |
| GOI | Gene of interest |
| hdNPC | Human degenerated nucleus pulposus cell |
| HEK293T | Human embryonic kidney 293T |
| hESC | Human embryonic stem cell |
| IBD | Inflammatory bowel disease |
| IL | Interleukin |
| IL-1Ra | Interleukin-1 receptor antagonist |
| iPS | Induced pluripotent stem |
| iPSC | Induced pluripotent stem cell |
| IPTG | Isopropylthio-β-galactoside |
| *L. lactis* | *Lactococcus lactis* |
| MESA | Modular extracellular sensor architecture |
| Mfp3 | Mussel foot protein 3 |
| mRNA | Messenger RNA |
| MSC | Mesenchymal stem cell |
| NF-κB | Nuclear factor-κB |
| NIR | Near-infrared |
| PLC | Polycaprolactone |
| PLGA | Poly (lactic-co-glycolic acid) |
| PLL | Poly-L-lysine |
| *S. cerevisiae* | *Saccharomyces cerevisiae* |
| SAP | Self-assembly peptide |
| sgRNA | Small guide RNA |
| shRNA | Short hairpin RNA |
| siRNA | Small interfering RNA |
| SynNotch | Synthetic Notch |
| TALEN | Transcription activator-like effector nucleases |
| TF | Transcriptional factor |
| TFF | Trefoil factor |
| TGF-β3 | Transforming growth factor-β3 |
| TLR5 | Toll-like receptor 5 |
| TNF | Tumour necrosis factor |
| TNF-α | Tumour necrosis factor-α |
| TSP-2 | Thrombospondin-2 |
| UCM | Upconversion material |
| UCNP | Upconversion nanoparticle |
| VHH | Variable fragments of heavy chain antibody |
| ZFN | Zinc finger nucleases |

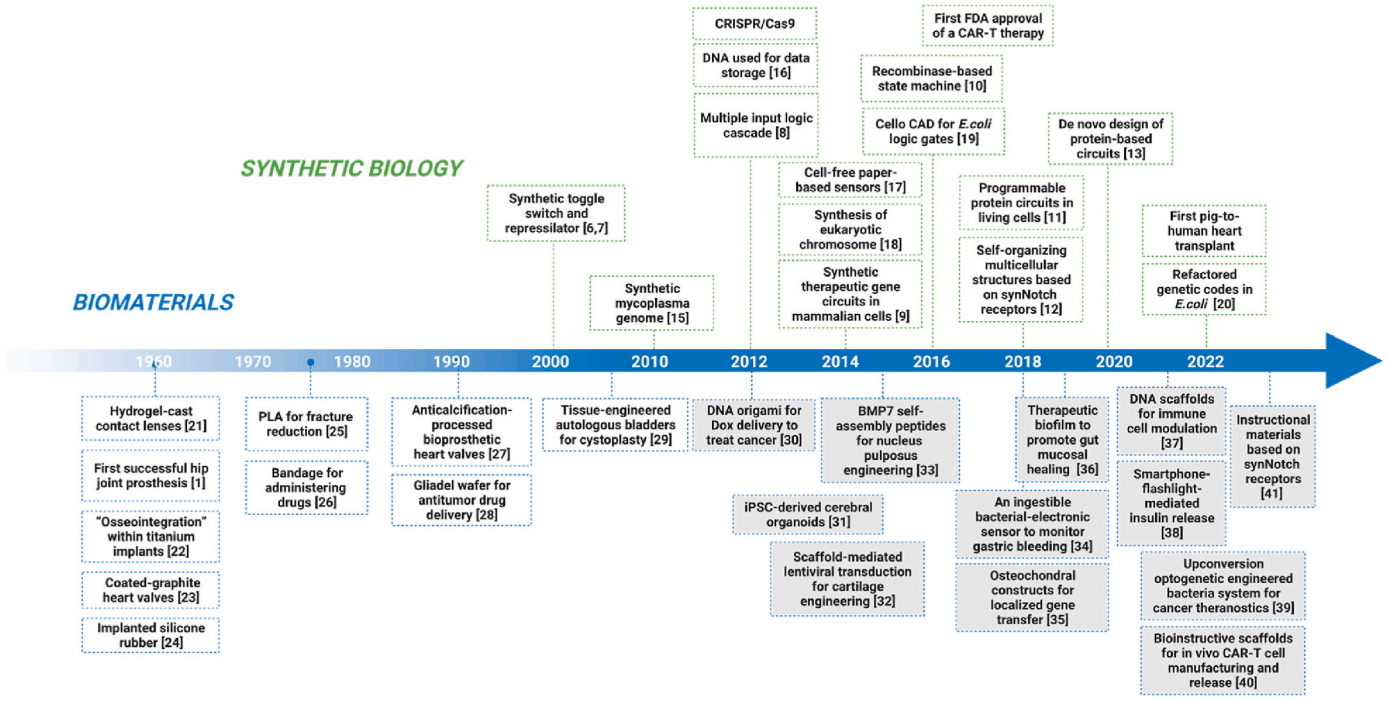


**Fig. 1.** Hallmarks in the development of synthetic biology and biomaterials [1,6,8–13,15–41]. Examples of synthetic biology-innovated biomaterials are shaded in grey. Created with BioRender.com.

**Table 1**
Examples of synthetic biology-innovated biomaterials.

| Types | Biomaterials | Purposes | Fabrication methods | Working mechanisms |
|---|---|---|---|---|
| Non-living materials | DNA origami for doxorubicin (Dox) delivery to treat cancer [30] | Drug delivery for cancer therapy | Intercalation of Dox to the structurally customized DNA nanotubes | Dox is a drug for breast cancer and can intercalate to DNA. Optimal delivery of Dox can be achieved by tuning the nanostructures of DNA origami used as the drug carriers. |
| | DNA scaffolds for immune cell modulation [37] | Cell immune modulation for CAR-T therapy safety improvement | Immobilization of synthetic DNA strands on the surface of poly (lactic-co-glycolic acid) (PLGA) polymeric particles and using it as an adaptable scaffold for loading bioactive molecules for immune modulation | DNA origami was used to functionalize the surface of PLGA particles as a platform for the tuneable loading of proteins. The scaffolds can be intratumorally injected and used for the surface presentation of priming antigens that activate CAR T cells driving local tumour clearance. |
| | BMP7 self-assembly peptides for nucleus pulposus engineering [33] | Nucleus pulposus tissue engineering | Fusing a self-assembly domain (RADA16-I) with functional domains of BMP7 | The RADA16-I self-assembly domain helps the peptides form into hydrogels after incubation. The BMP-7 domains have important effects on preventing disc degeneration. |
| | Scaffold-mediated lentiviral transduction for cartilage engineering [32] | Cartilage tissue engineering | Immobilizing lentivirus vectors on poly-l-lysine (PLL)-coated 4D woven polycaprolactone (PLC) scaffolds | Scaffold-mediated transduction enabled the expression of TGF-β3, which could induce chondrogenesis in this system. |
| | Osteochondral constructs for localized gene transfer [35] | Osteochondral tissue engineering | Engineering a two-layered scaffold to mimic the osteochondral structure and immobilizing the two layers with lentiviruses encoding two different growth factors (TGF-β3, BMP-2) respectively | Site-specific transductions were achieved by spatial confinement of lentiviruses. MSCs transduced on the top layer could overexpress TGF-β3 and induce chondrogenesis. While cells transduced on the second layer would overexpress BMP-2 and induce osteogenesis. |
| | CRISPR-responsive smart materials for diagnostics and programmable cargo release [42] | Diagnostics and cargo release | Incorporating DNA into materials as both a structural and information-encoding element. Incorporation of cargos (e.g. fluorophores, tethered molecules, physically entrapped large cargos) into the hydrogels. Synthesis of Cas12a guide RNAs (gRNAs) targeting the DNA sequence incorporated in materials. | The administration or presence of gRNAs in the environment can lead to Cas12a-mediated cleavage of the targeted DNA inside the materials. Depending on the structural function of targeted DNA, the cleavage can result in different changes in the material properties, such as the release of quenched fluorophores from the scaffolds or large cargo release caused by bulk hydrogel depolymerization. |
| Hybrid living materials | Yeast biosensor for dipstick tests [43] | Detection of pathogenic fungi | Engineered yeast biosensors loaded on filter papers | The engineered yeast can generate a red pigment once the detection of fungal mating peptides. |
| | An ingestible bacterial-electronic sensor to monitor gastric bleeding [34] | Detection of gastric bleeding | Engineered bacteria combined with electronic devices | Bacteria were engineered to detect heme and report a luminescent output to electronics which could wirelessly transmit the results. |
| | Smartphone-flashlight-mediated insulin release [38] | Treatment for type 1 diabetes | Light-generating devices combined with microencapsulated designer cells | The smartphone's flashlight was used as a stimulation to trigger the secretion of insulin of subcutaneously implanted designer cells. |
| | Bioelectronics for button-controlled insulin release [44] | Treatment for type 1 diabetes | Electro-sensitive designer cells were encapsulated in a piezoelectric device | The piezoelectric materials can transform mechanical compression into electrical stimuli, that can activate the designer cells to produce insulin. |
| | Upconversion optogenetic engineered bacteria system for cancer theranostics [39] | Cancer therapy | Engineered bacteria connected with upconversion nanoparticles | The tumour-targeting bacteria *Escherichia coli* Nissle 1917 (EcN) were engineered to release tumour apoptosis-related inducing ligands through blue-light-inducible self-lysis. The upconversion nanoparticles attached to the bacteria can be used for time-resolved imaging of tumours to improve diagnostic accuracy. |
| | Upconversion nanoparticle-enhanced bacterial cancer therapy [45] | Cancer therapy | Optogenetically engineered bacteria combined with upconversion nanoparticles | The upconversion nanoparticles can transform near-infrared photoirradiation to blue light, which subsequently could activate the engineered bacteria to release molecules promoting tumour killing. |
| | A photoautotrophic living material for skin wound healing [46] | Wound healing | Hydrogel encapsulation of engineered microbial consortium | The engineered consortium consists of *Synechococcus elongatus* for producing sucrose by photosynthesis and another heterotrophic engineered bacterium for long-term secretion of therapeutic molecules. |
| | Implants for self-regulating anti-cytokine therapy in rheumatoid arthritis [47] | Rheumatoid arthritis | Engineered stem cells encapsulated in agarose rod implants | The designer cells encompassed a self-regulating gene circuit that can detect and suppress inflammation. The agarose hydrogel was designed into a rod shape for subcutaneous injection. |
| | Bioinstructive scaffolds for in vivo CAR-T cell manufacturing and release [40] | Cancer therapy | Fabrication of macroporous scaffolds conjugated with activation cues (anti-CD3 and anti-CD28 antibodies). Loading the scaffolds with T cells and retroviruses encoding a CD19-specific CAR. | The bioinstructive scaffolds serve as biocompatible CAR-T cell factories in vivo. |

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In this review, we specifically focus on the applications of synthetic biology in theranostics and explore how it is driving the development of advanced biomaterials. We begin with a concise introduction to provide fundamental concepts in synthetic biology, aiding readers in understanding how user-defined functions can be programmed. Subsequently, we provide an overview of cutting-edge applications to offer insights involved in the development of this exciting field and envision future opportunities at the intersection of synthetic biology and biomaterials.

## 2. Programming biomaterials with user-defined functions

Synthetic biology employing a "design-build-test-learn" cycle aims to assemble biological systems using standardized bio-parts in a "plug-and-play" manner. User-defined functions can be engineered at different levels, including single biological molecules, signalling pathways, cells, and multicellular communities. In this section, we will focus on how functions can be programmed at the single-cell level (Fig. 2). We will provide an overview of how user-defined functions can be achieved by different engineered chassis harbouring well-designed gene circuits [49,50]. It is worth noting that while cell engineering is a major approach in synthetic biology, functions can also be encoded in cell-free systems as mentioned, though further details will not be discussed here.

### 2.1. Choosing a chassis

Chassis, in the context of synthetic biology, refers to cell hosts providing essential resources for gene circuits to function. A diversity of chassis has been exploited for biomaterials development, which encompasses different advantages and disadvantages (Table 2). Microorganisms such as *Escherichia coli (E. coli)* and *Saccharomyces cerevisiae (S. cerevisiae)* that have rapid growth rates, a large collection of genetic parts and tools, and well-studied metabolic background, are widely used chassis for biomaterials fabrication [51]. In clinical applications, microbial chassis are usually selected for their commensal or probiotic properties to mitigate the potential outcomes arising from direct administration or exposure to the human body [36,52].

Mammalian cells offer a more direct and safer choice for biomedical applications. Several types of cell-based therapies have been established and are commercially available. These include hematopoietic progenitor cells which are used for hematopoietic and immunologic reconstitution, chimeric antigen receptor (CAR)-T therapies that have received approval as advanced treatments for tumours, and the use of an allogeneic cell-laden scaffold known as STRATAGRAFT® to treat thermal burns [58]. In addition to their established safety profile, mammalian cells possess more sophisticated expression systems compared to prokaryotic chassis. These systems facilitate proper protein folding and post-translational modifications, resulting in the production of biologically active proteins [59]. In fact, certain mammalian cells inherently recognize disease-related biomarkers, simplifying the engineering process [60].

### 2.2. Coding a function

Gene circuits are composed of biological molecules that interact with each other and utilize the host cell's machinery to perform specific functions. Different chassis may possess distinct sets of machinery, which influence the selection of gene elements for designing gene circuits. Nevertheless, the underlying engineering strategies are similar. A stimulus-responsive cell function can be abstracted and divided into three steps: input perception, signal processing, and output actuation (Fig. 3A). Inputs represent the diverse chemicals or physical stimuli in the environment that cells are programmed to sense, such as chemical molecules, light, heat, pH, etc. These external signals can be naturally sensed or detected by synthetic sensing modules, which are usually membrane-bound receptors (e.g. synthetic Notch (SynNotch) receptors), ion channels (e.g. mechanosensitive ion channels), or intracellular regulators (e.g. reverse tetracycline-controlled transactivator protein (rtTA), light-inducible activators based on cryptochrome 2 (CRY2)/calcium- and integrin-binding protein 1 (CIB1) heterodimers). These modules subsequently can activate intracellular signalling via different mechanisms such as dimerization, conformation change, enzymatic cleavage, or the generation of secondary signals [61]. This leads to further steps of signalling processing or regulating output actuation directly. Complex gene circuits functioning as decision-makers, besides the sensing and actuation modules, often contain a layer of processing modules that analyse and integrate the activated signalling pathways. In electronics, circuits consist of distinct electronic components to undertake different signal processing. Similarly, synthetic biology has developed biological counterparts, such as gene switches, logic gates, oscillators, memory elements, filters, thresholders, and amplifiers, enabling multiplexing processing [62–64]. For example, logic gates that integrate multiple inputs according to a defined logic algorithm can be used to improve the specificity of disease recognition [65]. The final step, output actuation, represents the outcome of a function. The step is usually mediated by gene transcription and regulated by the input-process results. This could lead to diverse outcomes, such as cell migration [66,67], proliferation inhibition [68], differentiation, protein expression and secretion [69], gene knock-in or knock-out [70,71], etc.

The first-generation of synthetic gene circuits were inspired by the gene regulation model of prokaryotic operons, which consists of three

**Table 1** (*continued*)

| Types | Biomaterials | Purposes | Fabrication methods | Working mechanisms |
|---|---|---|---|---|
| | Instructional materials based on synthetic Notch (synNotch) receptors [41] | Orthogonal interactions between cells and biomaterials | Functionalization of biomaterial surfaces with specific ligand-capturing motifs. Engineering cells with synNotch receptors responding to the targeted soluble ligand. | When the soluble ligands are added to the niche, functionalized biomaterial surfaces can capture the ligands and activate artificial functions of engineered cells through corresponding synNotch receptors. This allowed the engineering of orthogonal interactions between cells and biomaterials. |
| Self-organizing living materials | iPSC-derived cerebral organoids [31] | Cerebral organoid models | In vitro organoid culturing treated with proper media compositions | Cells have capabilities of self-organization. iPSCs are multipotential and can differentiate along specific lineage in proper conditions. |
| | Self-organizing multicellular structures based on synNotch receptors [12] | Complex morphology of multicellular structures | Engineering cells with synNotch receptors | A synthetic cell-cell communication was created by engineering cells with synNotch receptors. |
| | Therapeutic biofilm to promote gut mucosal healing [36] | Gut mucosal healing | Genetic engineering of EcN | Engineered EcN can colonize the damaged sites and secrete the curli-fused trefoil factors that promote epithelial restitution. |
| | Functionalized biofilm to prevent pathogen infection in the gut [48] | Infection prevention of gut | Genetic engineering of EcN | Engineered EcN can colonize inside the gut and produce curli fibres fused with single domain antibodies against enteric pathogen virulence factors. |

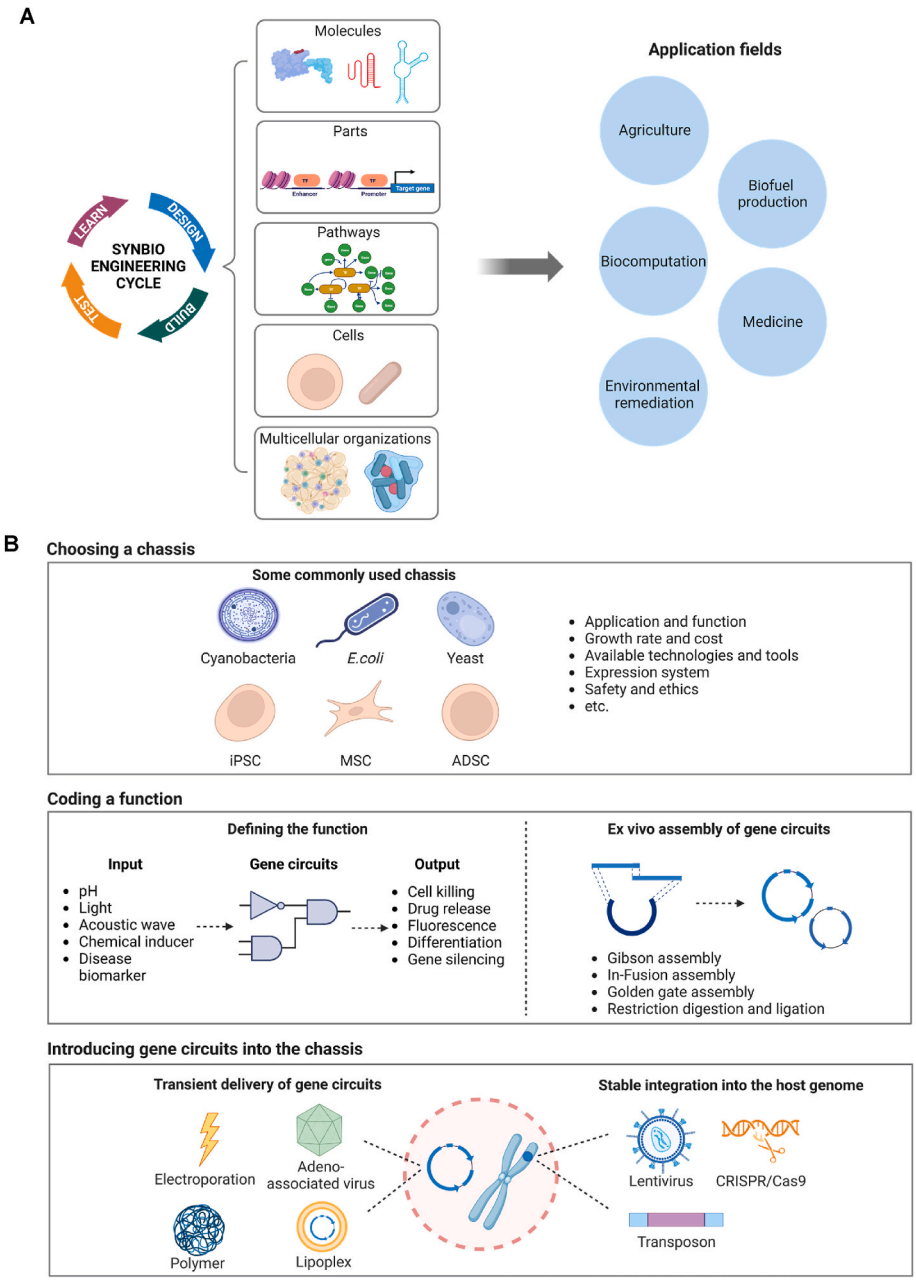


**Fig. 2.** Programming user-defined functions using synthetic biology. (A) User-defined functions can be programmed in different hierarchical levels of biological systems to solve problems in diverse fields. (B) Steps for programming a user-defined function at the level of a single cell. Created with BioRender.com.

classes of gene elements: regulatory genes, operators (*cis*-regulatory elements targeted by regulator), and regulated genes [78]. For example, the *lac* operon contains an operator (*lacO*) between the target genes (*lacZYA*) and their promoter (Fig. 3D). The operon also includes a *lacI* gene encoding a regulator that can bind to *lacO* and inhibit the transcription of *lacZYA*. However, in the presence of lactose or its synthetic analogue, isopropylthio-β-galactoside (IPTG), the regulator can be released from the operator allowing gene transcription. By replacing *lacZYA* with genes of interest, biologists can design a simple gene circuit for controllable gene transcription triggered by IPTG induction. Numerous prototyping gene circuits have been developed based on such operon-inspired semi-artificial regulation systems (e.g. Tet-on, Tet-off) to achieve target gene expression in response to specific chemical inducers. However, the limited number of these gene elements and chemical inducers impede the construction of more complex gene circuits for diverse applications. Therefore, synthetic biologists have developed more building blocks for gene circuit construction. For example, synthetic receptors, with customized extracellular or intracellular domains have been developed to rewire pre-existing signalling pathways [73,74]. Optogenetics elements, mechanosensitive ion channels, and voltage-gated ion channels have been applied to design gene circuits regulated by light, mechanical compression, and electrical stimulus, respectively. A diversity of artificial transcriptional regulation systems has been designed based on the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system, that gives flexible regulation of gene expression [79]. By wiring and coordinating these modules for input sensing, processing, or output actuation, gene circuits can be constructed for different functions. And gene circuits can be orthogonally built-in chassis cells or be partially introduced relying on certain signalling pathways of chassis.

Besides, as function complexity increases, computer-aided design based on mathematical models is becoming more and more important

**Table 2**
Some representative chassis in the development of theranostic biomaterials [35,40,43–48,53–57].

| Types | Advantages | Disadvantages | Representative chassis | Characteristics and typical applications |
|---|---|---|---|---|
| Bacteria | Rapid cell growth and proliferation<br>Small and simple genomes<br>Abundant methodologies for genetic engineering<br>High expression level of recombinant proteins<br>Cost-efficient production and up-scaling | Risk of immunogenicity and pathogenicity<br>Limited post-translational modifications | *Escherichia coli* Nissle 1917 | Probiotic, gut colonization, tumour targeting capability<br>Engineered biofilms to prevent enteric pathogens [48]<br>Bacteria-based cancer therapy enhanced by upconversion materials [45] |
| | | | *Komagataeibacter xylinus* | Bacterial cellulose production<br>Enhanced bacterial cellulose production by employing synthetic ribosome binding sites [53] |
| | | | *Lactococcus lactis* | Lactic acid secretion, cytokines regulating inflammation<br>Bacteria-activated hydrogels for diabetic wound healing [54] |
| | | | *Synechococcus* spp. | Photosynthesis<br>Oxygen-releasing scaffolds to promote dermal regeneration [55]<br>Hydrogel-encapsulated engineered microbial consortium as a photoautotrophic living material for wound healing [46] |
| Fungi | Well-studied genomes<br>Robustness and cost-efficient production | Risk of immunogenicity and pathogenicity | *Saccharomyces cerevisiae* | Living sensors to detect fungal pathogens [43] |
| Mammalian cells | Desirable post-translational modifications<br>Inherent capabilities of perceiving some disease-related biomarkers<br>Increasing methods for genetic engineering<br>The successful clinical translation of several cell-based therapies | Complex genomes<br>Difficulties during genetic engineering<br>Low recombinant protein expression level<br>High-cost production<br>Cell identity and heterogeneity | Mesenchymal stem cells | Differentiation potency, immunomodulation<br>Scaffold-instructed transduction of MSCs for osteochondral interface repair [35] |
| | | | Induced pluripotent stem cells | Differentiation potency, risk of tumorigenicity, development of patient-specific cell lines<br>Light-inducible neuronal differentiation [56]<br>Cell-laden hydrogels for self-regulating treatment of rheumatoid arthritis [47] |
| | | | Human embryonic kidneys 293T | An immortalized cell line, high transfection efficiency<br>Subcutaneous implants enabling push button-controlled insulin release [44] |
| | | | T lymphocytes | Major components of the adaptive immune system<br>CAR-T therapies combined with bio-instructive implantable scaffolds [40] |
| Plant cells | Photosynthesis<br>Abundant natural metabolites<br>High yield<br>Free from ethical issues | Complex genomes<br>Difficulties during genetic engineering | *Nicotiana tabacum* | Heterologous production of hyaluronic acid [57] |

for expediting the engineering cycle. Numerous computational tools have emerged in past decades, that take various functions including visualization and automated design of gene circuits [19], kinetic simulation of synthetic modules [80], and structural prediction of macromolecules [81]. These tools can provide a theoretical evaluation of designs before wet lab building and reduce laborious bench work. Moreover, machine learning which does well in complex puzzles has also been employed in coping with sophisticated biological systems [82]. In this regard, models can be built by training instead of based on acknowledged theories, which could be a promising supplement to conventional approaches.

### 2.3. Introducing gene circuits into the chassis

Once gene circuits are assembled ex vivo, the next step will be installing the functions inside the chosen chassis. Gene circuits can be set up either in a permanent fashion by integrating them into the genome, or in an episomal form that functions transiently and gets lost over time. The choice of setting up strategy depends on the intended function and application (Fig. 2).

For applications that require functions to be operational for a limited period, transient transfection strategies are sufficient. Various methods based on different mechanisms have been developed to facilitate the delivery of gene elements across the cell membrane and into the nucleus. These methods include physical/mechanical techniques such as electroporation, microinjection, and sonoporation as well as lipid or non-lipid-based chemical vectors (e.g. cationic lipids, polymers, and peptides). Viral-based transient transductions using adenovirus, adeno-associated virus, or herpes virus vectors are also commonly employed.

However, to achieve stable and long-term functions, gene circuits are usually inserted into the genome of the chassis. Methods for stable transfection include viral vectors (e.g. retrovirus and lentivirus), tamed transposons (e.g. Sleeping Beauty and PiggyBac), site-specific recombinases (e.g. FLP/FRT system and Cre recombinase), and other genome editing techniques like Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas systems. These approaches can be used individually or in combination, allowing for random insertions or site-specific integration of DNA fragments of different sizes, as well as replacement or deletion of native fragments within the chassis genome.

It is worth noting that while the discussed cellular functions are primarily encoded with recombinant DNA and operate based on transcriptional regulation, other biological molecules, such as RNA (mRNA, siRNA, shRNA) can also carry out designed functions [64]. Gene circuits based on these molecules offer different advantages compared to DNA-based ones. For example, mRNA-based engineering enables direct protein translation in the cytosol, eliminating the risk of insertional mutagenesis.

## 3. Synthetic biology innovated biomaterials for theranostics

Synthetic biology offers a diverse array of tools and techniques for the precise engineering of biomaterials, affording meticulous control over their properties and functions. This capability heralds new horizons in the field of theranostics. Harnessing the potential of biological systems, synthetic biology boasts several unique advantages over traditional chemical approaches. These include: 1) Faster and economic production: Synthetic biology allows for the metabolic engineering of cell factories, enabling faster and more cost-effective production of biomaterials. 2) Tailored or novel molecules: By genetically engineering cells, synthetic biology provides the means to produce tailored or novel molecules that can either be artificially synthesized or produced by the cells. 3) Functionalizing hybrid materials: Synthetic biology enables the programming of cells as functional mediators, which can be

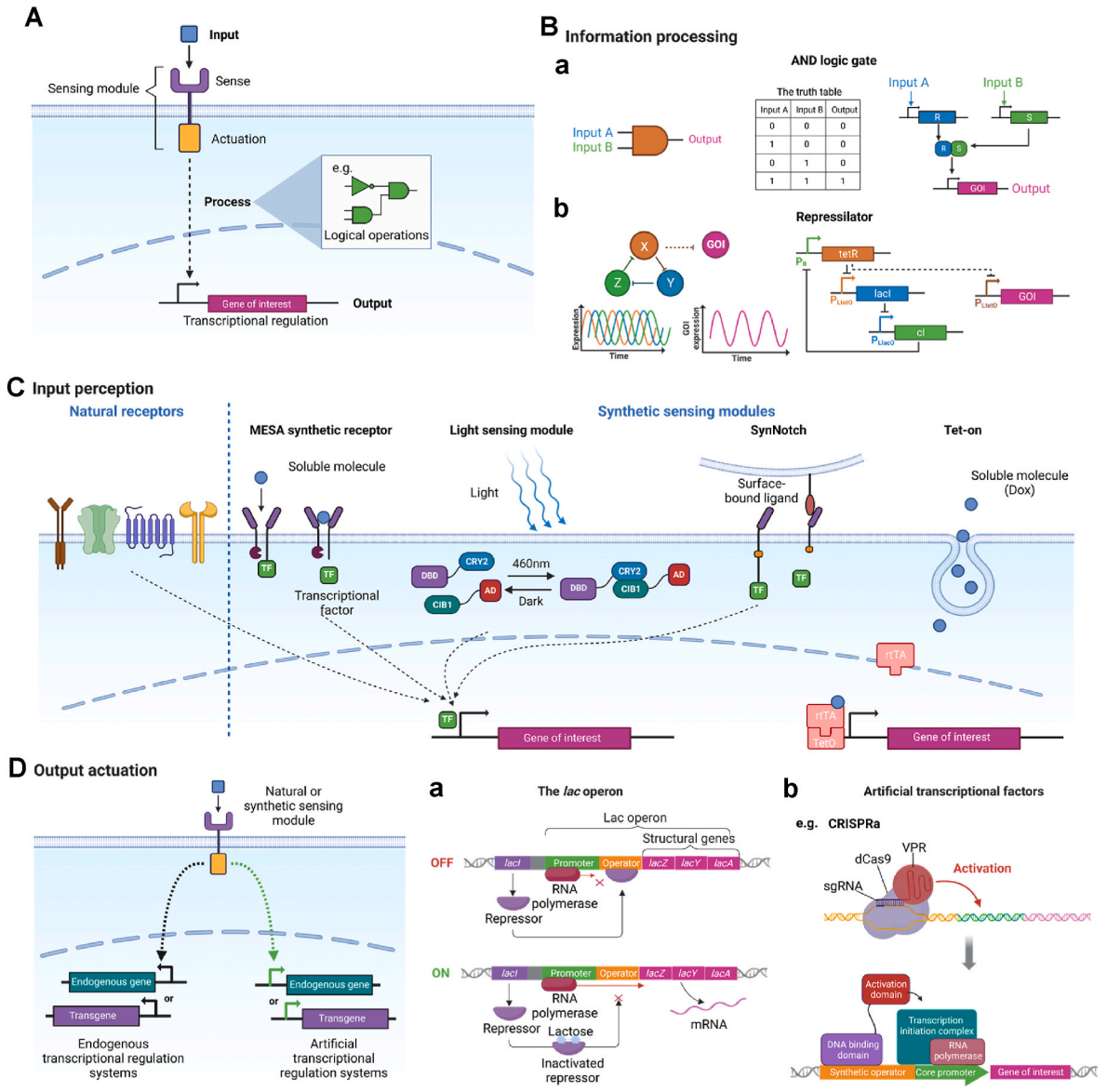


**Fig. 3.** Schematic illustration of how cellular function can be coded using gene circuits. (A) A cellular function can be abstracted into steps of input perception, information processing, and output actuation. (B) Two examples of gene modules involved in information processing. (a) A two-input AND gate that produces output only in the presence of both inputs [72]. (b) A biological repressilator that generates an oscillatory production of the output. (C) Input perception is mediated by either natural or synthetic receptors that lead to intracellular signalling activation. Examples of synthetic receptors include a modular extracellular sensor architecture (MESA) receptor, a light switch, a SynNotch receptor, and a Tet-on system [73,74,75,76]. (D) Output actuation can be mediated by transcriptional regulation of either endogenous genes or transgenes. (a) The *lac* operon derived from prokaryotic cells can be used for inducible expression of the gene of interest. (b) A CRISPR-based activation system is given as an example of an artificial transcriptional regulation method [77]. Created with BioRender.com.

incorporated into well-designed containers, creating hybrid living materials with enhanced functionalities. 4) Complexity and controllability of self-organizing biomaterials: Synthetic biology adds complexity and controllability to self-organizing living biomaterials, allowing them to better adapt to specific applications.

In this section, advanced biomaterials are grouped into three categories within the context of theranostics: 1) non-living biomaterials, this category encompasses bioactive components produced from engineered cell factories such as self-assembly peptides and programmed decellularized extracellular matrix, and gene-activated matrices; 2) hybrid living materials, here we include living biosensors, bioelectronic devices, and optogenetic controlled hybrid biomaterials systems; 3) self-organizing living materials composed of living cells and cell-secreted matrices, this category involves tailored biofilms and self-organizing multicellular systems.

### 3.1. *Synthesizing customized non-living biomaterials*

Traditionally, biomaterials have relied on natural compositions produced by living cells, such as nucleotides and proteins, which can be synthesized cell-free or harvested and isolated from cell-based production. These materials can be used as single components (e.g., collagen, fibrin, hyaluronic acid) or as a combination of multiple compositions (e.g., decellularized tissue). However, synthetic biology offers the opportunity to introduce new characteristics to these natural materials or create entirely new counterparts through rational design. Here are some advances in the field of biomedical applications using customized non-living biomaterials.

### 3.1.1. Rational design of self-assembly peptide (SAP) for tissue regeneration

Synthetic biology, aided by computational tools and mathematical models, enables the rational design of biological molecules in terms of their structure and function. This includes nucleotides (e.g. DNA/RNA aptamers to display selective affinities [83,84]), peptides, and proteins (e.g. chimeric proteins to elicit multivalent protections [85]). SAPs have gained significant attention from biomaterials scientists due to their biocompatibility, biodegradability, extracellular matrix (ECM) mimicking features, and the engineering potential offered by the infinite combinations of amino acids and sites for chemical modifications [86]. SAPs can be developed by redesigning existing peptides or protein motifs mined from nature, or by designing entirely new peptides [87]. For instance, functionalized SAPs conjugated with bone morphogenetic protein-7 (BMP-7) have been used for tissue engineering of the nucleus pulposus in intervertebral discs (Fig. 4A) [33]. Nanofibrous hydrogel scaffolds formed by these functionalized peptides promoted human degenerated nucleus pulposus cells (hdNPCs) proliferation, migration, and extracellular matrix secretion. In addition to their application in tissue engineering, SAP biomaterials have also been engineered to serve as novel antimicrobial agents and drug carriers for different uses [88], although further details will not be provided in this context.

### 3.1.2. Programmed decellularized extracellular matrix with improved properties

Decellularized extracellular matrices (dECM) have gained popularity as biomaterials for tissue engineering. These naturally derived materials exhibit distinct advantages over synthetic ones, such as having some growth factors favourable to wound healing, taking endogenous degradation pathways, and low immunogenicity [90]. However, the tunability of dECM is limited due to the natural sources of their production [91]. Synthetic biology offers the ability to genetically manipulate the components of ECM, allowing for more flexible control over its properties. For example, Morris et al. created tuneable dECM hydrogels derived from decellularized skins of thrombospondin-2 (TSP-2) knockout mice, possessing altered mechanical properties and increased regenerative effects [92]. Additionally, induced pluripotent stem (iPS) technology can reprogram somatic cells to become pluripotent cells, which can be re-differentiated into desired cell lineages. This approach has been employed to produce patient-specific dECM for the treatment of diabetic foot ulcers (DFU), leading to enhanced ECM production and wound healing (Fig. 4B) [89]. The researchers utilized biopsied cells from DFU patients and reprogrammed them into rejuvenated fibroblasts (iDFU) with enhanced production of ECM containing a rich composition of wound healing factors. The rejuvenated fibroblasts were seeded on collagen–glycosaminoglycan scaffolds (CG) and decellularized, creating personalized scaffolds (D-iDFU). CG, D-iDFU as well as decellularized DFU fibroblast-functionalized scaffolds (D-DFU) were compared on their abilities to induce regenerative responses in patient-matched DFU fibroblasts and unmatched controls (healthy fibroblasts). It showed the D-iDFU outperformed the CG and D-DFU groups in promoting fibroblast proliferation as well as glycosaminoglycan (GAG) and collagen accumulation. Interestingly, D-iDFU seeded with matched pathological DFU fibroblasts showed even higher fibroblast proliferation as well as GAG, collagen I, and collagen IV content compared with the scaffolds seeded with healthy but unmatched fibroblasts. This might suggest an improved therapeutic effect resulting from the personalized approach.

### 3.1.3. Gene-activated biomaterials matrices

Gene-activated biomaterial matrices represent another avenue in synthetic biology to enhance the functionality of artificially synthesized materials. In this approach, gene circuits carried by viral or non-viral vectors can be incorporated into scaffolds before or after fabrications, thereby providing spatially patterned instructive cues for cells following implantation. An illustrative example is the fabrication of a bi-layered construct designed for osteochondral interface repair (Fig. 4C) [35].

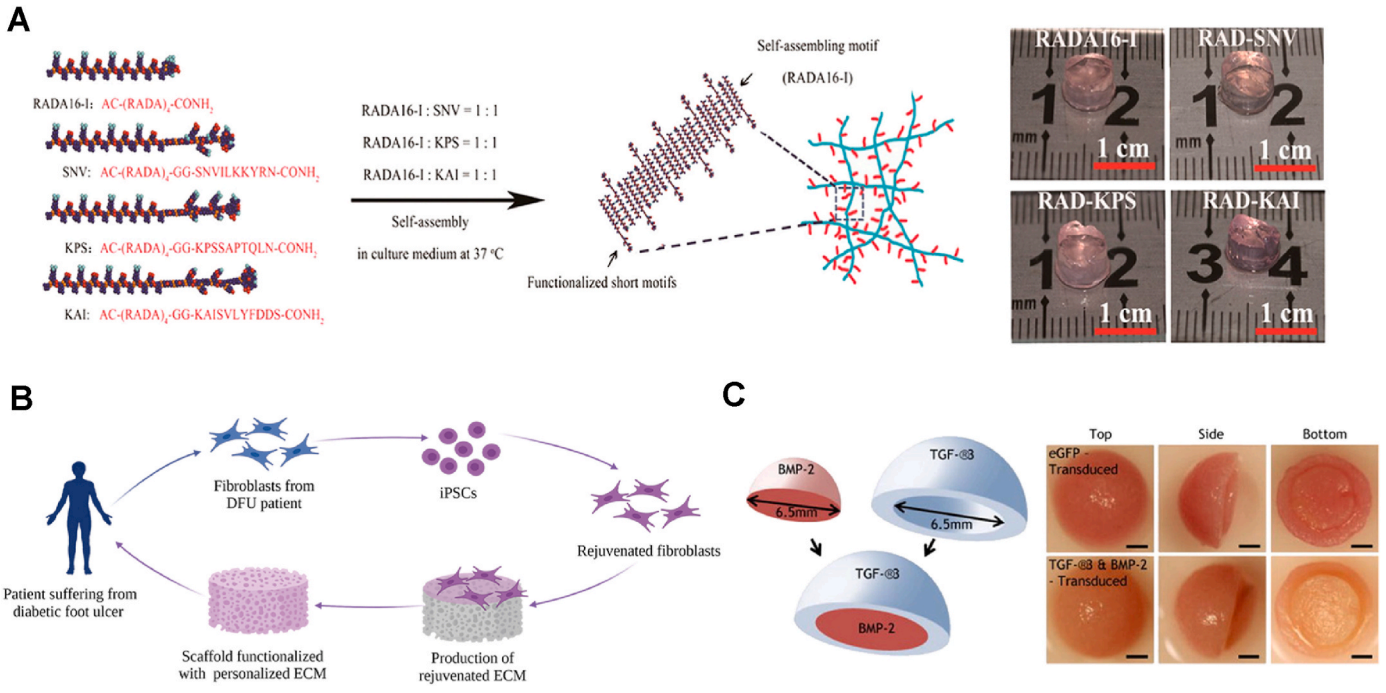


**Fig. 4.** Examples of non-living biomaterials innovated by synthetic biology. (A) Self-assembly peptides functionalized with BMP7 domains for tissue engineering of nucleus pulposus. The functional SAPs are designed by fusing a self-assembly motif (RADA16-I) with different peptides derived from BMP7 (SNV, KPS, KAI). When mixed with RADA16-I peptides, the SAPs can form into hydrogel-like scaffolds which can be used for tissue engineering of the nucleus pulposus. Adapted with permission from Ref. [33]. (B) Illustration of rejuvenated DFU fibroblasts used to produce personalized extracellular matrices that can be used to functionalize scaffolds providing precise DFU treatment [89]. Created with BioRender.com. (C) A gene-activated construct with spatially patterned lentiviral particles for osteochondral engineering. The top layer was immobilized with particles for TGF-β3 transduction, while the second layer was designed for BMP-2 transduction, providing cells with instructions for bidirectional differentiation. Scale: 2 mm. Reprinted with permission of Elsevier [35].

The outer shell of this construct was immobilized with lentiviral particles containing the gene encoding transforming growth factor-β3 (TGF-β3), while the inner core was engineered to deliver the gene encoding bone morphogenetic protein-2 (BMP-2). Through site-specific transduction of mesenchymal stem cells, this approach enabled simultaneous chondrogenic and osteogenic differentiation within their respective scaffold layers, effectively mimicking the structure of a native osteochondral interface.

### 3.2. Engineering hybrid living materials

In recent years, significant attention has been directed towards the development of responsive biomaterials. These materials exhibit controlled changes triggered by either endogenous physiological cues within microenvironments (e.g. pH, redox potential, enzymes, and hydrolysis), or external physical stimuli (e.g. light, acoustic sounds, and magnetics). Such materials have found applications in stimuli-triggered drug release [93], such as an insulin-releasing patch designed based on the glucose-sensing properties of phenylboronic acid [94], and have been used to create dynamic microenvironments for guiding cell fate [2]. However, as the demand for precise and complex input-output relationships in medical scenarios continues to grow, purely chemical approaches may no longer be the preferred choice for engineering responsiveness.

Instead, synthetic biology-driven innovations in cell-laden materials offer more promising alternatives by harnessing the responsive capabilities of living systems and merging them with the complementary features of abiotic materials. In this context, engineered cells can directly mediate stimuli-response functions while being encapsulated within abiotic materials that not only shield cells from immune attacks but also provide a conducive living niche. The incorporation of different materials facilitates multifaceted enhancements of theranostic functions beyond cell maintenance (Fig. 5). For example, engineered cells can be formulated with hydrogels for 3D bioprinting, enabling precise cell patterning and the fabrication of complex 3D architecture. Abiotic components can be introduced to transmit, interpret, and improve the input-and-output signals, thereby complementing the functions of cells. Moreover, materials can be employed to provide instructions to cells by incorporating input cues like conjugated ligands and soluble inducers. In response, the cells produce outputs that dynamically alter the properties of the materials. These highly specialized theranostic biomaterials exist in diverse forms and serve various purposes, expanding their potential applications not only in the field of biomaterials but also in the realm of cell-based therapies.

#### 3.2.1. Hybrid living materials for ex vivo diagnostics

Synthetic biology approaches have been used to create biosensors that can detect disease-specific biomarkers and generate interpretable outputs, such as pigments or fluorescence. These living sensors can be combined with supportive materials to enable various diagnostic strategies. For instance, a modular yeast biosensor has been developed for the low-cost detection of pathogenic fungi and the platform can be potentially scalable to global surveillance of other pathogens (Fig. 6A) [43]. The biosensor, designed as a ready-to-use product, contains genetically modified *S. cerevisiae* that generates a red pigment readout upon sensing pathogen-derived peptides. Samples such as urine and serum can be easily detected by a one-step dipstick assay, without specialized equipment or trained personnel. Additionally, bacterial sensors have been combined with 3D printing technology to create living tattoos that can stick to human skin and sense different chemical inducers, potentially enabling wearable diagnostics in the future [95].

#### 3.2.2. Bioelectronic devices for wireless health monitoring and point-of-care therapies

Implantable bioelectronic devices that provide wireless theranostic strategies have gained increasing interest in recent years. Synthetic biology-programmed theranostic cells can be coupled with electronic devices, such as smartphones, through wireless communication technologies, allowing real-time health monitoring and point-of-care treatments. For instance, an ingestible bacterial-electronic device has been

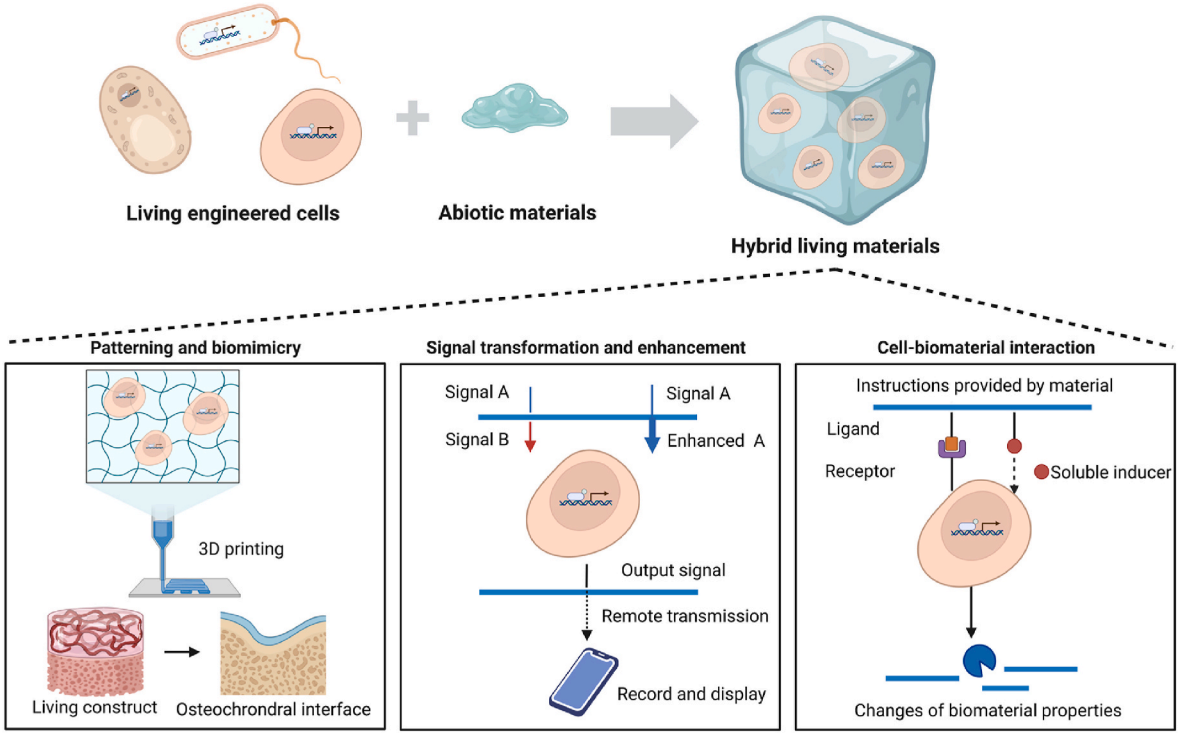


**Fig. 5.** Schematic illustration of hybrid living material compositions and multi-perspective functional enhancements mediated by abiotic materials. Created with BioRender.com.

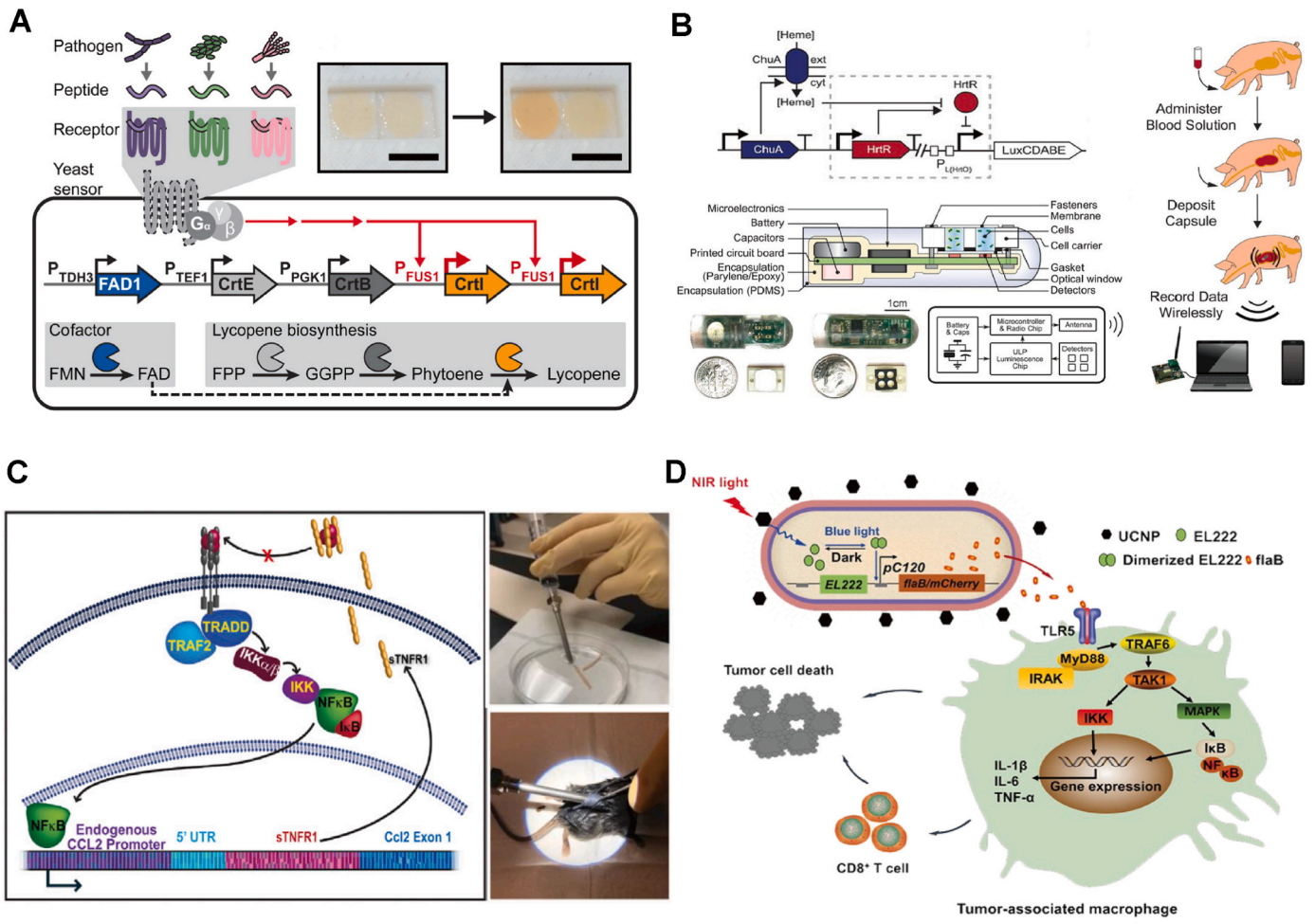


**Fig. 6.** Examples of hybrid living materials applied in ex vivo (A) and in-vivo (B) diagnostics as well as therapeutics (C, D). (A) A yeast sensor engineered to detect fungal peptides and generate a visible pigment output. The sensor consists of highly specific fungal receptors that respond to mating peptides produced by pathogenic fungi. Activation of the receptors results in the biosynthesis of red lycopene pigment. The biosensor allows paper-based dipstick assay and results can be interpreted according to the colour. FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate. Scale bars, 0.5 cm. Reprinted with permission of AAAS [43]. (B) A bioelectronic device designed for real-time gut bleeding monitoring. The device consists of blood-sensing cells producing a luminescent output and electronic components transforming luminescence into digital signals and transmitting results wirelessly. Reprinted with permission of AAAS [34]. (C) Illustration of a close-loop gene circuit for autonomous inflammation suppression (left). In this circuit, the presence of tumour necrosis factor-α (TNF-α) can activate sTNFR1 expression under a Ccl2 promoter, which can then block the TNF-α in the microenvironment and alleviate inflammation. The engineered Ccl2-IL1Ra cells were encapsulated in rod-shaped hydrogel constructs for injectable delivery and better maintenance (right). Figure adapted with permission of Elsevier [96] and © 2023 Collins et al. [47]. (D) A bacteria-based cancer therapy enhanced by upconversion nanoparticles (UCNPs). In this approach, the UCNPs convert NIR light into a blue light and activate engineered bacteria to secret flagellin B (flaB), a potential activator of the Toll-like receptor 5 (TLR5) pathway. This can lead to the tumour-killing effects of tumour-associated macrophages by secreting proinflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and TNF-α. Adapted from Ref. [45] with permission of John Wiley and Sons.

designed for monitoring gastrointestinal tract bleeding (Fig. 6B) [34]. Heme-responsive bacteria detect blood and produce luminescence, which is interpreted by a low-power luminometer chip. The device converts luminescence into digital signals and transmits results wirelessly outside the body for display and recording. This platform enables continuous health tracing for patients. Bioelectronics can also be designed for point-of-care treatment, such as a coin-sized bioelectronic device for wireless glycaemic control in type 1 diabetic mice [38]. Electrosensitive human β cells programmed for electro-triggered insulin release were integrated into the device, allowing for electro-triggered normoglycemia restoration. Recently, the team has repurposed the electrosensitive cells to develop a subcutaneous-implantable device that enables insulin release through push-button control [44]. This innovative design utilizes a piezoelectric material to simplify the complex electronic components responsible for pulse generation. By converting mechanical compression into electrostimulation, the piezoelectric material streamlines the device's functionality. While the aforementioned therapeutic cells have been engineered to respond to electrical stimuli, advancements in synthetic biology have paved the way for cellular programming to detect various physical inputs, such as light, acoustic sounds, and magnetics. Consequently, these emerging possibilities hold great potential for the future development of wireless-controlled bioelectronic therapies.

### 3.2.3. *Disease-responsive biomaterials for self-regulated drug delivery*

In addition to wireless-controlled therapies, there is a growing trend toward designing self-sufficient treatments, where therapeutic cells autonomously perform medical interventions in response to disease-specific biomarkers. In this regard, numerous cell devices have been developed as drug depots for self-regulated delivery. Unlike systems that rely on externally administrated inducers, these devices can adjust the secretion of drugs autonomously, eliminating the need for precise timing and dosage considerations. For example, cells encapsulated in alginate-PLL-alginate beads have been engineered and intraperitoneally

implanted in mice to sense and suppress inflammation [63]. The cells contain a nuclear factor-κB (NF-κB) responsive element-based sensor that activates downstream signalling in response to inflammation. The signals are then processed by an amplifier and a positive feedback loop-based thresholder, leading to the expression of anti-inflammatory effectors. On-demand drug delivery has also been applied to restore homeostasis in endocrine or metabolic disorders, such as hyperuricemia and Graves' disease [97,98], offering an alternative to repeated drug injections. Additionally, self-regulated drug delivery can be used to regulate the microenvironment for tissue repair. By inserting the soluble type 1 tumour necrosis factor receptor (sTNFR1) or interleukin-1 receptor antagonist (IL-1Ra) gene downstream of an inflammation-responsive promoter (the promoter of chemokine (C–C) motif ligand 2 (Ccl2)), the engineered cells (Ccl2-sTNFR1 or Ccl2-IL1Ra) can deliver anti-inflammatory sTNFR1 or IL-1Ra in the presence of inflammation, facilitating cartilage repair in osteoarthritic joints (Fig. 6C) [96]. In follow-up work, Ccl2-IL1Ra cells were seeded in agarose hydrogel constructs made from 3D-printed molds, which can be injected subcutaneously by a needle. These rod-shaped hydrogel constructs containing Ccl2-IL1Ra cells showed excellent effects in mice K/BxN serum transfer arthritis models. The agarose hydrogel can maintain better encapsulation and long activity of Ccl2-IL1Ra cells. Besides, the injectable delivery avoided invasive surgery and implantation [47].

#### 3.2.4. Upconversion materials enhanced optogenetic-controlled cell therapy

One notable application of materials in cell therapies involves the use of upconversion materials to facilitate optogenetically controlled treatments. Light plays a crucial role as an inducer in cell-based therapies, providing high precision and tunability. However, the limited tissue penetration of blue-light activation, which is commonly used in optogenetic systems, restricts its biomedical applications. To overcome this limitation, upconversion materials (UCMs) have been developed to convert high-penetrating near-infrared (NIR) light into blue light. This enables the activation of blue-light-based optogenetic systems using NIR light, significantly expanding their potential applications. For instance, UCM-cell systems can be utilized to generate real-time probiotic interventions for regulating the microbe-gut-brain axis or to provide precise immunotherapy against tumours (Fig. 6D) [45,99].

It should be noted that the role of materials in living hybrids extends beyond providing shelter or preventing cell leakage. Despite the aforementioned examples, there exists a significant research gap in the field of material-enhanced cell therapies.

### 3.3. Programming self-organizing living materials

Self-organizing living materials, composed of living cells and cell-secreted matrices, play a crucial role in the field of theranostics. These materials encompass human or animal-derived organs and tissues, offering autologous, allogenic, or xenogeneic grafts for transplantation. Additionally, artificial multicellular structures like organoids also fall into this category. Microorganisms, on the other hand, can form biofilms that embed cells within self-produced matrices, or even compose living consortia consisting of multiple microbial populations. In this context, synthetic biology enables the programming of functions at a collective level, extending beyond individual cells, by determining what is produced and how the entity responds. It also allows for the engineering of the consortia, influencing communication between units within the entity and defining their organization, population, and evolutionary dynamics.

#### 3.3.1. Repurposed biofilms for gastrointestinal health

Curli fibers, natural self-assembling materials produced by Gram-negative bacteria as the major component of biofilms, serve to mediate adhesion and protect bacteria in harsh environments. The intersection of synthetic biology and biomaterials has led to the customization of biofilms for various applications, such as biocatalysts, environmental remediation, gradient mineralization, and also medical care [100]. One common engineering strategy involves fusing the amyloid-like self-assembly monomers, known as CsgA, with distinct proteinaceous domains that possess therapeutic functions. For instance, Néel S. Joshi et al. described an oral-administrated biofilm to treat inflammatory bowel disease (IBD) [36]. They genetically engineered a probiotic *E. coli* strain Nissle 1917 (EcN) to produce curli fibers with tethered therapeutic domains called trefoil factors (TFFs). TFFs are a family of small cytokines secreted by mucus-producing cells that promote epithelial reconstitution. However, direct oral administration of TTFs often results in unsatisfactory therapeutic outcomes as the molecules primarily accumulate in the small intestine [36,101]. To achieve on-site interventions, TFFs were genetically modified to be fused with CsgA and displayed as part of the EcN biofilm. Following oral administration, the biofilm colonized IBD-affected sites, leading to ameliorated disease activity in a colitis mouse model. More recently, the team repurposed the EcN biofilm for the prevention of diarrheal diseases (Fig. 7A) [48]. The CsgA monomer was fused with various antibody domains (VHHs) targeting different enteric pathogens, transforming the EcN biofilm into a versatile platform for pathogen sequestration.

Furthermore, biofilm can be programmed with complex stimuli-responsive properties. For instance, An et al. programmed a living glue system capable of autonomously repairing blood-leakage sites (Fig. 7B) [102]. The recombinant glue consists of nanofibers assembled from CsgA monomers fused to mussel foot protein 3 (Mfp3) and enhanced by tyrosinase post-modification. This system was developed using two *E. coli* strains that operated in a division of labor. To enable blood-responsive glue production, a heme-sensing gene circuit was introduced into the CsgA-Mfp3 synthesizing *E. coli* strain, leading to heme-triggered glue and Acyl-homoserine lactone (AHL) production. AHLs are commonly used signal molecules in synthetic biology for intercellular communication. In this design, it activates gene circuits in another *E. coli* strain that produces tyrosinase to improve glue adhesion. As a proof of concept, the system was tested with a microfluidic device simulating gastrointestinal (GI) bleeding and demonstrated blood-triggered production of living glue, effectively sealing the damaged sites. Beyond GI health management, *Lactococcus lactis* (*L. lactis*) biofilms have also been programmed to modify abiotic material surfaces and engineer instructive microenvironments that regulate stem cell activities, including cell adhesion and differentiation by presenting different cues [100,103,104].

#### 3.3.2. Programming complex self-organizing multicellular systems

Tissue engineering and regenerative medicine aim to create artificial replacements for dysfunctional tissues or organs, alleviating the burden of donor shortage. To recapitulate native structures and functions, current studies predominantly employ a top-down strategy, fabricating biocompatible materials that mimic tissue-specific ECM properties and seeding specific cell types within or onto the materials post-fabrication. Precise spatial control of cell types can be achieved through approaches such as 3D bioprinting, enabling the fabrication of complex tissue architectures [105]. This top-down strategy focuses on designing the extracellular environment to contain the proper signals for guiding the differentiation of stem cells toward the lineage of choice (Fig. 8). While this approach has been successful in designing various tissue-engineered constructs, precise control of cell differentiation remains a significant challenge.

In contrast, a bottom-up approach involving direct modulation of stem cell behaviour through genetic modification holds promise for developing more effective cell-laden constructs for tissue engineering. For instance, optogenetic tools have been developed, providing high-resolution spatial and temporal control of various cell activities, such as neurogenesis [56], myogenesis and angiogenesis [106], and proliferation-differentiation transition [107]. Furthermore, a bottom-up engineering approach that leverages the self-organizing nature of cells

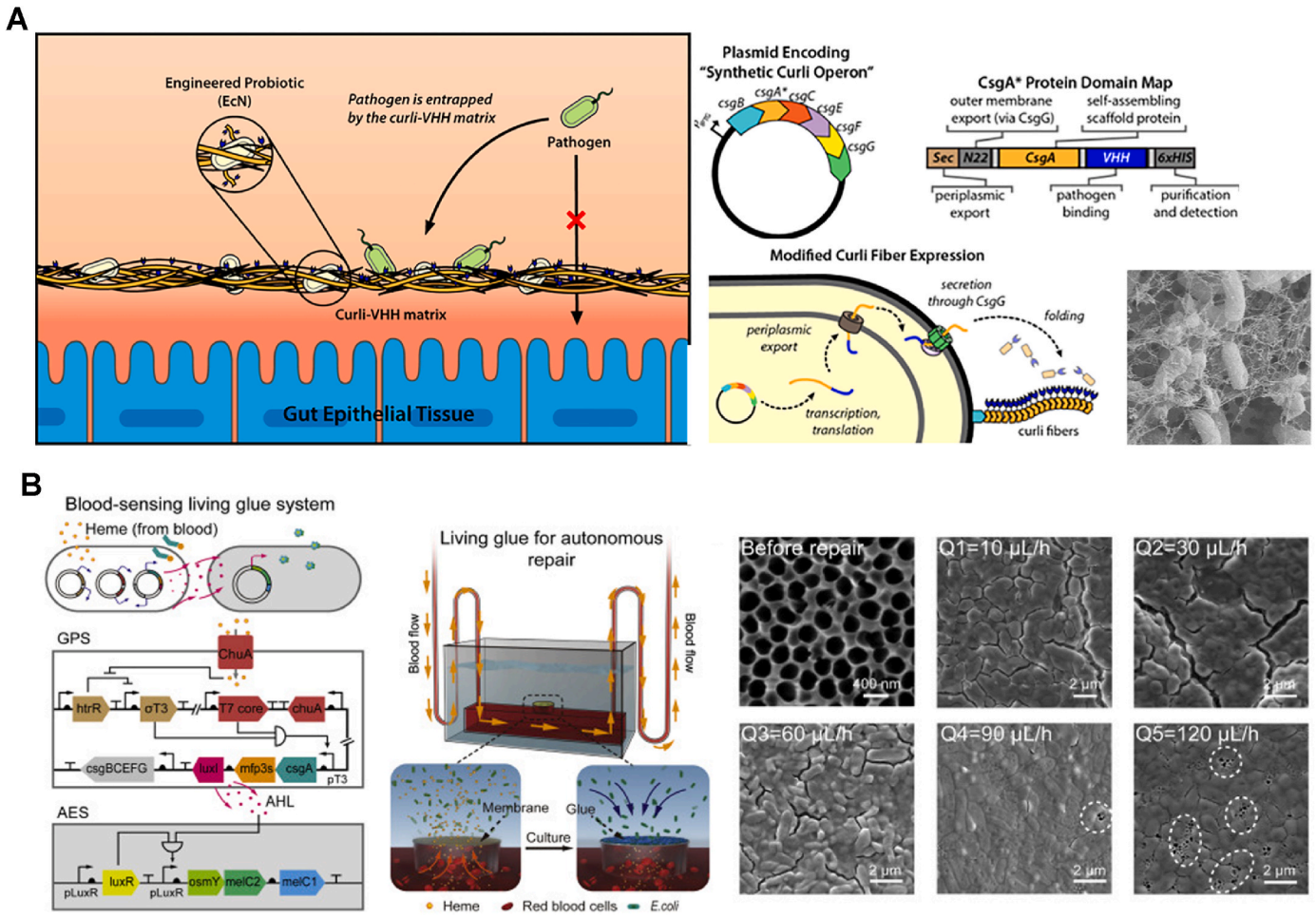


**Fig. 7.** Biofilms repurposed by synthetic biology for different theranostic applications. (A) EcN biofilms were engineered to protect the gastrointestinal tract from pathogen infection. The CsgA domain of curli fibres is fused with VHH domains specifically binding to pathogens. © 2022 Gelfat et al. [48]. (B) A biofilm-based living glue system was designed to sense blood and seal the leakage. The system consists of two genetically engineered *E. coli* strains one can sense blood and produce functionalized CsgA monomers, and the other produces molecules enhancing the adhesive (left). As a proof-of-principle, the system was assessed in a microfluidic device simulating gastrointestinal bleeding (middle). The engineered *E. coli* could produce biofilm and seal the porous leakage sites (right). Reprinted with permission of Elsevier [102].

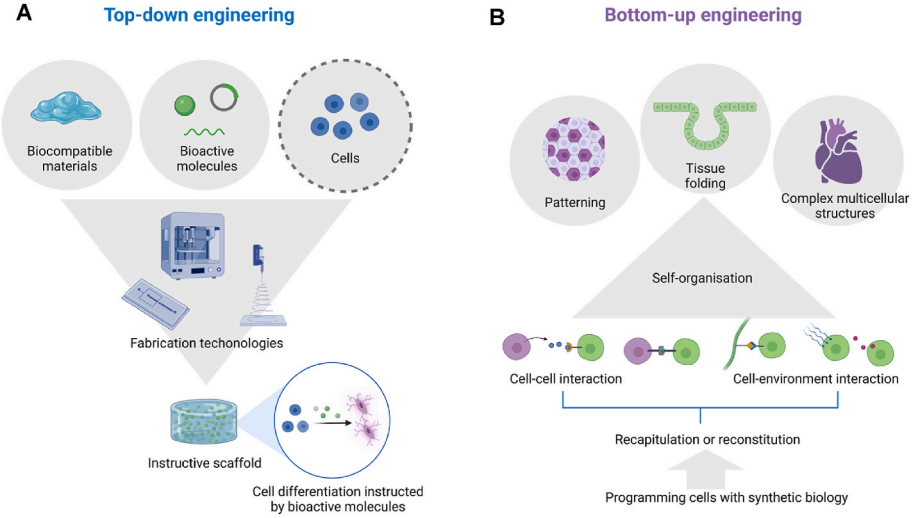


**Fig. 8.** Schematic illustration of how tissue constructs can be developed either by a top-down (A) or a bottom-up (B) engineering approach. Created with BioRender.com.

has also made significant progress. Organoids, 3D multicellular structures that recapitulate native features of specific tissues or organs, can be derived from adult mammalian tissue or differentiated from induced pluripotent stem cells (iPSCs) [108]. Different soluble morphogens can be added to the culture media to direct a fraction of progenitors toward specific lineages for organoid formation. However, organoids developed by this approach often lack the structural and functional complexity of native tissues. Synthetic biology provides powerful tools to add complexity to multicellular organizations. For example, it can build gene circuits to reconstitute or recapitulate cell-matrix and cell-cell communication that naturally occur during embryonic development or tissue self-renewal [109]. Additionally, it can introduce external controls of cellular behaviours, as mentioned earlier in the context of optogenetics. Although bottom-up construction of artificial organs still faces challenges, state-of-the-art synthetic biology approaches can lay the foundation for future advancements in this field.

## 4. Challenges and opportunities

### 4.1. Biosafety and bioethics

Working with living materials containing genetically engineered cells or microorganisms raises significant biosafety concerns, potentially posing risks to both human health and the environment [109]. While several CAR-T therapies based on patient's own cells have achieved commercial availability, the potential for allogeneic or xenogeneic cell leakage from theranostic biomaterials following implantation could trigger immune rejection and worsen the patient's conditions [110,111]. Immortalized cell lines such as human embryonic kidney 293T (HEK293T) cells are widely used due to their efficient transfection capabilities, enabling the establishment of various artificial theranostic functions [9,69,98]. However, the unlimited proliferation of these cells in vivo poses a tumorigenesis risk that impedes their clinical translation [112]. Similarly, iPSCs have been employed in fabricating biomaterials for tissue repair and regeneration, capitalizing on their differentiation potential [56,113]. Nevertheless, their clinical application has been hindered by genomic instability and the potential for malignancy [114,115]. To address the concerns of cell leakage and uncontrolled proliferation of theranostic biomaterials used in implantation, strategies have been explored in the form of physical containment. For instance, hydrogels, with their highly hydrated polymer networks, are widely used for cell encapsulation. To enable sustained containment, hydrogel properties, including pore size and mechanical characteristics, can be optimized to control substance diffusion and minimize cell leakage [116,117]. For example, living materials made from fragile hydrogels are easy to break and may result in cell leakage under repeated deformation. In response to this, Liu et al. presented a method to create stretchable and robust hydrogel-elastomer hybrids capable of hosting programmed cells [117]. To prevent unconstrained cell proliferation and function within the human body, theranostic devices can be designed for easy retrieval after usage [118]. Safety switches that induce theranostic cells' apoptosis or halt their functions immediately can also be used to maintain control [119,120].

Another critical safety concern revolves around horizontal gene transfer and antibiotic resistance resulting from genetically engineered microorganisms. This poses substantial risks to the genetic stability of ecosystems and may lead to antibiotic-resistant "superbugs" in natural settings, with synthetic biology potentially accelerating this process [121]. To address these concerns, various strategies including physical encapsulation, active anchorage of bacteria to matrices [122], auxotrophy, and kill/suicide switch, may be applied solely or in combination to confine the genetically engineered microorganism and address environmental safety concerns [123].

Moreover, interdisciplinary research in this field may raise ethical questions concerning biomaterials, synthetic biology, and cell-based therapy. For example, theranostic biomaterials involving living cells prompt inquiries about the source of these cells. Ethical controversies have arisen surrounding stem cell research, such as the ethical validity of destroying embryos to obtain human embryonic stem cells (hESCs), informed and voluntary consent for cell donation, and the risks and benefits of experimental intervention [124]. Synthetic biology, which involves creating or redesigning biological systems, has been the subject of debate for decades, with concerns about "playing god" and apprehensions regarding the creation of new life forms, posing unknown threats to public health, the environment and life itself [125]. Some countries have taken steps to regulate the potential risks associated with synthetic biology by enacting laws and regulations [121].

As novel biomaterials continue to emerge through multidisciplinary collaboration, and as new participants join the field, researchers must be attuned to these critical issues beyond their specific research domains. Additionally, the development of standards is imperative for categorizing diverse theranostic biomaterials, and the establishment of new legislative and regulatory constraints is crucial for assessing the biosafety and ethics of research and the clinical translation of these innovative biomaterials.

### 4.2. Up-scaling production and quality control

Addressing the challenge of up-scaling production and maintaining quality control in the context of living biomaterials is pivotal. As these materials become more complex and diverse, it is essential to develop scalable manufacturing processes capable of consistently yielding large quantities. A common strategy for up-scaling biomaterial production involves the utilization of large-scale fermentation processes and bioreactors. In this approach, microorganisms engineered via synthetic biology with enhanced resilience to metabolic and environmental stresses are cultured in these systems to produce biomaterials.

Furthermore, cell-free synthetic biology approaches can be employed to avoid the limitations of cell-based production and can be adapted for use in remote, low-resource settings. By harnessing cell extracts containing the necessary cellular machinery, it is possible to produce biomaterials in a more scalable and controlled manner. Adiga et al. developed an automated, portable Bio-MOD system utilizing lyophilized Chinese hamster ovary cell extracts to yield an end-to-end cGMP-quality manufacturing process in under 9 h. Through this technology, critical biomolecules like His-tagged granulocyte-colony stimulating factor and erythropoietin can be reliably produced at the point of care [126]. Moreover, developing modular synthetic biology components and standardized parts can facilitate the scaling process. These components can be easily assembled and optimized to create large-scale production systems. For instance, Wang et al. devised a comprehensive synthetic biology toolbox named BIOPOLYMER, enabling the engineering of four distinct anthracyclinone pathways within the host *Streptomyces coelicolor* M1152, resulting in titers of 103 mg/L of nogalamycinone production [127].

In terms of quality control during the production of theranostic biomaterials, the implementation of advanced analytics and monitoring systems is essential. These systems provide real-time monitoring of critical process parameters, such as pH, temperature, and nutrient levels. Furthermore, advanced analytical techniques like mass spectrometry and roman spectrometry can be harnessed to evaluate biomaterial quality throughout the scaling process [128]. Robust measures must be implemented to ensure the consistency and reproducibility of the living materials. For engineered mammalian cells deployed in regenerative medicine, the translation is also largely hindered by the limited production capacity of current cell expansion regime and quality control. To tackle this issue, Du et al. developed 3D dissolvable microcarriers for large-scale expansion of cells. These microcarriers possess a porosity rate exceeding 90 % and offer control over particle sizes ranging from 50 to 500 μm, thereby enabling 3D biomimetic cultivation. This approach is well-suited for adherent cell culture and the efficient harvesting of cells and cell products [129].

### 4.3. Exploring cell-material interaction

Exploring and controlling the interaction between cells and the material matrix is crucial for the development of hybrid living materials. Empirical studies have found the properties of the material, such as porosity, elasticity, and stiffness, can influence cell signalling and behaviour, including adhesion, proliferation, and differentiation. While the mechanisms underlying these correlations remain elusive, leaving a lot of opportunities to explore. Synthetic biology enables more precise control of the interface between cells and materials. It can program cells using material properties as inputs to activate the gene circuits [41]. However, unrevealed cell-material interactions may lead to crosstalk between gene circuits and non-specific signalling, causing failures of user-defined functions. Understanding the mechanisms behind and orchestrating correlated signal pathways promises the rational design of functionalities. On the other hand, the precise and tuneable features of synthetic biology opened new opportunities for deep understanding or controlling of cell-material interaction. Artificial biofilms, for example, beyond theranostics have also been used to engineer cell-material interface, mimicking an ECM-resembled dynamic niche to provide spatiotemporal cues impacting cell behaviours [103,104,130]. In brief, further research is needed to optimize these interactions and create biomaterials that can effectively modulate cellular responses.

## 5. Outlook

The intersection of synthetic biology and biomaterials holds great promise for the development of advanced biomaterials with self-regulated and programmable properties. These new biomaterials can overcome the limitations of traditional materials and better interact with complex tissue microenvironments. We envision that the delineation of the functionalities of biomaterials empowered by synthetic biology will become increasingly blurred in the foreseeable future, giving rise to a new class of integrated biomaterials that possess multiple functions, including sensing, diagnostics, self-regulated drug delivery and the capability for self-removal. Furthermore, the advances made in system biology and quantitative mathematical modeling are poised to furnish robust tools for engineering living therapeutic biomaterials that can provide precise real-time feedback, potentially even executing specified computational operations in the foreseeable future. By fostering collaboration between the synthetic biology and biomaterials communities, we can catalyse ongoing laboratory innovation, ultimately paving the way for transformative advancements within the biomaterial domain. This interdisciplinary approach holds the potential to revolutionize biomaterials research, ultimately contributing to the realization of personalized medicine in the future.

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## Ethics approval and consent to participate

Not applicable.

## CRediT authorship contribution statement

**Xiang Wang:** Conceptualization, Writing – original draft. **Qianyi Liang:** Data curation, Writing – original draft. **Yixuan Luo:** Data curation, Formal analysis, Writing – original draft. **Jian-Wen Ye:** Supervision, Writing – review & editing. **Yin Yu:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Fei Chen:** Conceptualization, Funding acquisition, Supervision, Validation, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

There are no conflicts to declare.

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