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Synthetic biology-driven microbial therapeutics: Integrated platforms for disease diagnosis and intervention

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ABSTRACT: Synthetic biology made the design of microbes as versatile therapeutic platforms possible, thereby transforming bacteria and synthetic microbial communities into programmable systems for health applications. This review summarizes recent progress in engineered bacterial therapeutics, emphasizing their roles in metabolic compensation, immune modulation, targeted delivery, and diagnostic sensing. Engineered strains have advanced from early proof-of-concept designs to sophisticated circuits that integrate environmental cues, respond to external stimuli, and achieve spatiotemporal control of therapeutic outputs. Similarly, synthetic microbial communities strategies provide ecological stability, functional redundancy, and customizable community interactions, offering advantages over single-strain interventions. Together, these approaches demonstrate a shift from conventional probiotics toward rationally designed microbial therapeutics that restore physiological balance and actively intervene in disease processes. Ongoing efforts address challenges, including colonization efficiency, biosafety, and regulatory adaptation, whereas emerging frameworks integrate multimodal sensing, intelligent feedback control, and clinical translation. This review emphasizes their potential to reshape future strategies for disease prevention, intervention, and personalized medicine by situating microbial therapeutics within the broader landscape of health engineering.

KEYWORDS: synthetic biology, microbial therapeutics, disease intervention, engineered bacteria, synthetic microbial communities

1 Introduction

The human commensal microbiota, particularly the gut microbiome, has been popularly recognized as a crucial regulator of host physiological homeostasis and disease development. Trillions of microorganisms collectively form a dynamic, symbiotic system with the host by participating in diverse processes such as nutrient metabolism, immune maturation, and neural regulation. Microbial dysbiosis has been closely associated with chronic disorders, including inflammatory bowel disease (IBD), obesity, and diabetes, and with cancer, neuropsychiatric conditions, and infectious pathologies. This understanding has guided the development of therapeutic strategies that target or employ microbes, among which engineered bacteria and synthetic microbial communities are emerging as highly promising approaches.

Conventional microbiota-based therapies, including probiotics and fecal microbiota transplantation (FMT), have exhibited therapeutic benefits in certain diseases but are limited by unclear composition, poorly defined mechanisms, low colonization efficiency, and high interindividual variability. These limitations have prompted the exploration of synthetic biology as a means to rationally engineer microbial systems with predictable behaviors and improved functional precision. Advances in synthetic biology have laid the foundation for developing controllable and predictable microbial therapeutic systems. Through precise genetic modifications, engineered bacteria can directionally express therapeutic molecules, sense pathological signals, and respond to environmental stimuli, thereby exhibiting properties of "living drugs". Concurrently, synthetic microbial communities are designed by integrating multiple functional modules through microbial ecology principles, emphasizing interspecies interactions and cooperative homeostasis, making them more suitable for addressing chronic, multifactorial, and complex disease models.

Building upon the growing understanding of host–microbiome interactions, recent breakthroughs in synthetic biology have helped in the development of programmable microbial therapeutics with improved precision and controllability. These advances incorporate engineered single strains and multispecies consortia within the broader context of disease prevention and intervention (Fig. 1). Subsequent sections focus on how such synthetic systems resolve key challenges in metabolic modulation, immune regulation, and clinical translation, thereby outlining future opportunities for health engineering.

2 Engineered bacteria: Expansion of functional modules and advancement of medical applications

Engineered bacteria are microorganisms that are modified through synthetic biology approaches to specifically express therapeutic factors *in vivo*, sense pathological signals, and execute targeted

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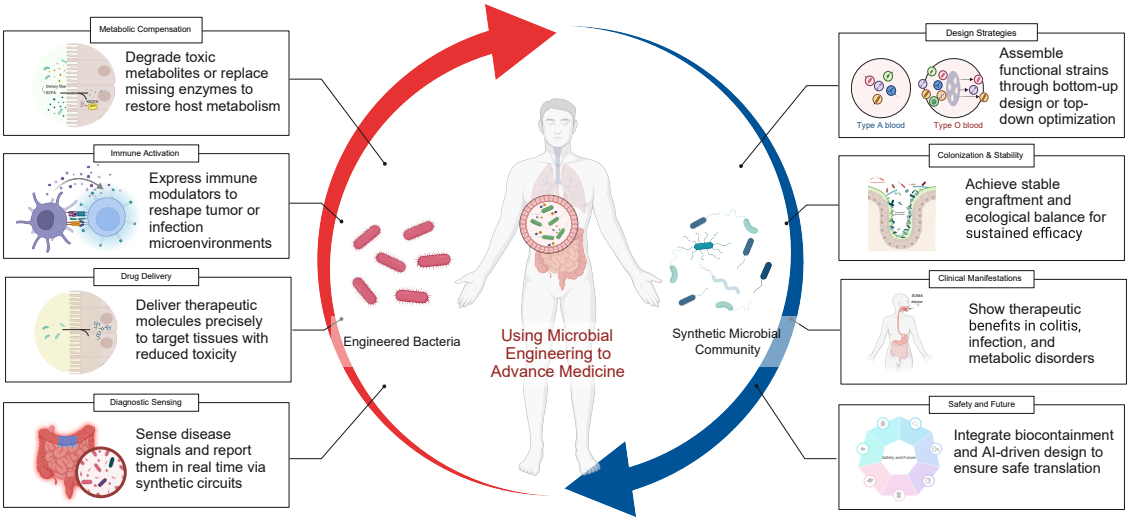

Figure 1 Engineered bacteria and synthetic microbial communities as emerging therapeutic modalities in health engineering. Created in https://BioRender.com

interventions [1] (Fig. 1). Engineered bacteria have been progressively organized into the following five major functional modules in recent years, with the maturation of genetic circuit construction, protein engineering, environment-responsive elements, and delivery platforms: metabolic compensation, immune activation, drug delivery, diagnostic sensing, and spatiotemporal regulation [2]. These functional advances have enabled their expanding applications in metabolic disorders, infectious diseases, cancer, and autoimmune conditions [3].

2.1 Metabolic compensation: Microbial metabolism to offset host deficiencies

Metabolic disorders frequently originate from defects in key metabolic enzymes or pathways. Conventional treatments, including dietary restriction or supplementation with metabolic intermediates, frequently fail to achieve precise control over drug concentrations or metabolic fluctuations [4]. By sustainably expressing functional enzymes and acting locally within the gut, engineered bacteria have emerged as an important complementary strategy for treating metabolic diseases [3].

Phenylketonuria (PKU) is a prototypical inborn error of metabolism caused by phenylalanine hydroxylase (PAH) deficiency, leading to impaired phenylalanine (Phe) conversion to tyrosine, systemic Phe accumulation, and consequent neurotoxicity, particularly intellectual disability in children [5]. Conventional management depends on lifelong low-Phe diets and medical foods, but long-term adherence is poor, and metabolic control is highly variable [6]. Sapropterin, a synthetic tetrahydrobiopterin, augments residual PAH activity in 20%–30% of mild to moderate cases but is limited by cost, lifelong use, and variable efficacy [7]. These shortcomings emphasize the need for mechanistically defined and sustainable alternatives.

To this end, a synthetic live biotherapeutic was developed by engineering *Escherichia coli* Nissle 1917 (EcN) to express phenylalanine ammonia lyase and an L-amino acid deaminase pathway, thereby enabling gut-localized Phe degradation into trans-cinnamic acid (TCA) and ammonia, with subsequent detoxification [8]. A first-in-human phase 1/2a trial revealed that SYNB1618 was well tolerated and exhibited dose-dependent pharmacodynamic activity, reflected by increased plasma TCA and urinary hippuric acid as strain-specific biomarkers [9]. The phase 2 Synpheny-1 study indicated that SYNB1618 and its optimized derivative, SYNB1934, markedly reduced plasma Phe, with SYNB1934 achieving up to 40% reduction in fasting Phe and enabling some patients to reach therapeutic targets without systemic toxicity [10]. However, clinical responses varied notably among individuals, probably due to differences in PAH genotype, diet, and gastrointestinal physiology. Moreover, the treatment effect wanes after dosing cessation, indicating limited durability and the need for chronic administration. No serious adverse events were reported; however, some participants discontinued owing to mild gastrointestinal symptoms, indicating that patient tolerance and adherence remain potential challenges. Further, these early trials comprised small sample sizes and short follow-up durations, and comparative effectiveness against existing therapies, such as sapropterin or pegvaliase, remains not established [10].

PKU represents a classic example of a metabolic disorder caused by an enzymatic deficiency. Engineered bacteria have been designed to compensate for such metabolic defects by degrading excess Phe in the gut. Cysteine is a central hub of the ferroptosis defense system in tumor cells. Its metabolic products supply precursors for the synthesis of glutathione (GSH), coenzyme Q10 (CoQ10), and tetrahydrobiopterin (BH4), thereby counteracting ferroptosis triggered by iron-dependent lipid peroxidation [11,12]. Oncogenic mutations in tumor cells, such as Kirsten rat sarcoma viral oncogene homolog (KRAS), commonly reprogram fatty acid, iron, and mitochondrial metabolism, which generates abundant lipid peroxides while creating a dependence on cysteine to maintain robust defense, thereby conferring high chemoresistance [13–19].

Slow onset and systemic adverse effects limit early attempts to attenuate ferroptosis defenses by dietary restriction of cysteine. Small-molecule ferroptosis inducers (e.g., erastin) can block Solute Carrier Family 7 Member 11–mediated cystine transport, but high toxicity, poor selectivity, and compensatory pathway activation constrain the efficacy [20–22]. Recently, inspired by natural sulfur-cycle mechanisms, researchers proposed using engineered bacteria to directly modulate cysteine metabolism [23]. Engineered strains degrade intra- and extracellular cysteine under the hypoxic tumor microenvironment by introducing cyst(e)inase (CGL) into nonpathogenic *Escherichia coli* MG1655, thereby efficiently dismantling ferroptosis defenses and markedly improving

ferroptosis sensitivity [24,25].

Further studies developed hypoxia-responsive engineered bacterial systems and employed red blood cell membrane camouflage to confer low immunogenicity and prolonged circulation. This design ensured stable intratumoral colonization and precise CGL expression while reducing systemic side effects, thereby achieving sustained cysteine depletion and ferroptosis induction in animal models [26]. Building on this, a composite platform combined engineered bacteria with nanomedicines. Strains obtained by directed evolution with improved cysteine/cystine uptake and degradation were integrated with liposomes carrying anti-angiogenic agents. This dual-hit strategy markedly disrupted redox homeostasis and improved ferroptosis induction by continuously weakening ferroptosis defenses while severing tumor blood supply [27].

These strategies extend to small organic acids beyond amino acid metabolism. Oxalate, a common dietary component, exemplifies how engineered bacteria are harnessed to overcome challenges in hyperoxaluria. Oxalate is an organic acid derived from diet and endogenous metabolism, which is widely present in various foods [28]. A portion is absorbed through the gastrointestinal tract and excreted as urinary oxalate (UOx) [29]. Enteric hyperoxaluria (EH), characterized by excessive oxalate absorption, leads to increased UOx, which contributes to formation of kidney stones and chronic kidney damage [30,31]. Current dietary interventions are limited, and approved drugs remain unavailable [32,33].

Gut microbiota plays a crucial role in oxalate metabolism. *Oxalobacter formigenes*, which uses oxalate as a carbon source, has been explored as a potential therapy for hyperoxaluria [34,35]. However, its use as a strict anaerobe faces production and colonization challenges [36]. In contrast, EcN, a noncolonizing probiotic, is a promising platform for engineered therapeutics owing to its safety, predictable pharmacodynamics, and complete clearance upon cessation [8,36]. EcN has exhibited potential in degrading toxic metabolites in the gastrointestinal tract.

Researchers developed SYNB8802, an engineered *E. coli* Nissle strain that degrades oxalate. SYNB8802 expresses oxalate decarboxylase, which reduces oxalate to formic acid and carbon dioxide in mouse models, thereby lowering oxalate absorption and renal excretion [37]. Mathematical modeling demonstrated the association between bacterial colonization, oxalate supply, and degradation efficiency, which provides a foundation for personalized microbial therapies. However, SYNB8802's noncolonizing nature requires continuous supplementation for efficacy, with limited clinical data available.

Remarkable advancements in 2025 were made with a new engineered therapeutic. Using *Phocaeicola vulgatus* as the chassis, researchers introduced a five-gene oxalate degradation pathway, thereby enabling controllable colonization and reversible clearance. This strain reduced UOx levels by 47% in rat models of EH and exhibited dose-dependent colonization, safety, and substantial UOx reduction in early human clinical trials [38]. The development of engineered bacterial therapeutics for oxalate metabolism is advancing, with controlled colonization strategies that improve efficacy and safety, thereby providing new treatment options for EH.

Similarly, the accumulation of ammonia poses severe risks to the host, and reprogrammed bacteria are being developed to provide efficient clearance in acute and chronic disease contexts. Intestinal ammonia originates mainly from glutamine deamidation in enterocytes and from colonic bacterial proteolysis and urease activity [39–41]. Normally, the hepatic urea cycle clears ammonia [42], but this is impaired in urea cycle disorders and cirrhosis, leading to hyperammonemia [42,43]. Patients demonstrate neurocognitive decline, with hepatic encephalopathy (HE) occurring in 30%–40% of cirrhotics [44–48]. Further, blood ammonia closely correlates with severity [49–52].

Conventional therapies—including rifaximin to suppress urease bacteria and lactulose to acidify the colon—reduce ammonia but suffer from poor compliance and side effects [53,54]. AST-120 adsorbs intestinal ammonia but demonstrates limited benefit [55]. Microbiota-based interventions, including low-urease consortia, delay ammonia generation and improve survival in mice [56]. Engineered *E. coli* strains convert ammonia to arginine, although *in vivo* efficacy remains unclear [57].

Synthetic biology has advanced this field: SYNB1020, an EcN strain, converts ammonia to L-arginine and effectively reduces hyperammonemia in mice and healthy volunteers, with good tolerability [58]. Extending this paradigm, large-scale analyses determined *Segatella copri* and its ammonia-assimilating gene *asnA* as protective for kidney function, and supplementation with *S. copri* or *asnA*-overexpressing *E. coli* mitigated chronic kidney disease progression [59]. Building on this, SYNB2081 integrated *asnA* overexpression and multistep ammonia pathways, thereby alleviating ammonia-induced renal injury in mice.

These cases emphasize how engineered microbes move beyond symptomatic relief to achieve sustainable metabolic reprogramming. They provide a versatile therapeutic paradigm for disorders ranging from rare genetic diseases to cancer and chronic organ dysfunction by acting locally in the gut while exerting systemic effects.

2.2 Immune activation: Mechanisms and strategies of engineered bacteria in modulating host immunity

Beyond serving as metabolic modulators, bacteria that are engineered through synthetic biology also function as potent immune activators within the host. In recent years, engineered strains have been extensively applied to modulate antitumor immunity, anti-infective responses, and autoimmune-related disease treatments [60]. Their immune-activating functions are primarily manifested in two ways: first, by expressing and delivering immunological effector molecules that directly stimulate immune responses; and second, by reprogramming the metabolism or functional states of immune cells to reshape the immune microenvironment [60,61].

Engineered bacteria are programmed to express tumor antigens, chemokines, cytokines, or antibody fragments, which enable continuous release of immune effectors and activation of innate and adaptive responses [61–63]. This approach has exhibited strong efficacy in multiple solid tumor models and is progressing from simple cytokine secretion toward designs that integrate microenvironmental sensing, precise triggering, and intracellular delivery [64]. Early efforts improved immune recruitment. For example, EcN engineered with CXCL16 (hCXCL16K42A) and CCL20 promoted cDC1–T cell crosstalk and tumor regression [65]. However, diffusion-limited strategies demonstrated poor control of distal lesions. To overcome this, bacteria were engineered to block immunosuppression. *E. coli* Nissle coexpressing anti-programmed death ligand 1 (PD-L1)/CD9 nanobodies with ZIF-8CHO–ICG generated ENZC, which releases nanobodies upon near-infrared

(NIR) irradiation to block PD-L1 on tumor cells and exosomes, thereby attenuating systemic suppression and reprogramming macrophages to M1 [66]. However, reliance on external light limited clinical feasibility. Endogenous tumor cues were then exploited. Hypoxia-responsive *E. coli* expressed anti-CD47 antibodies and carried redox-sensitive M-CSF liposomes, which improve macrophage activation and disrupt "don't eat me" signaling [67]. Further, microenvironment-triggered systems enabled combination therapy. *E. coli* Nissle with a radiation-responsive circuit secreted anti–TREM2 scFv in outer membrane vesicles (OMVs) in radiotherapy models, thereby reversing tumor-associated macrophage (TAM) polarization and synergizing with the PD-L1 blockade [68]. Beyond secretion, attenuated *Yersinia* with type III secretion was used to achieve intracellular delivery to translocate nanobodies into cells, thereby inhibiting BCR::ABL1 and inducing leukemia apoptosis [69].

Beyond expressing effector molecules, engineered bacteria enhance antitumor immunity by modulating immune cell states, which progress from immune initiation to tissue structuring, metabolic reprogramming, and homeostasis. Early work demonstrated the feasibility with commensals. *Staphylococcus epidermidis* engineered to express tumor antigens elicited antigen-specific $CD8^+$ T cells that infiltrated tumors and mediated cytotoxicity, thereby establishing a mild yet effective immune-priming platform [70]. However, single-signal activation lacked durability. Subsequent efforts structured the local immune architecture. In mucosal tumor models, attenuated *Salmonella typhimurium* VNP20009 expressing LIGHT via a lysis circuit activated HVEM (TNFRSF14) signaling on ILC3s, thereby driving mature tertiary lymphoid structures (mTLSs) that stabilized APC–lymphocyte interactions and sustained local responses [71]. However, metabolic constraints in tumors still limit immunity. To address this, immunometabolic modulation was pursued. A synthetic probiotic that releases lactate stabilized HIF-1α in dendritic cells, upregulated NDUFA4L2, reduced mtROS, and improved antigen presentation and T cell persistence under chronic stimulation [72]. Another strategy used high-tryptophan–producing *Clostridium butyricum* (L-Trp CB) that secretes tryptophan and butyrate, which counters indoleamine 2,3-dioxygenase-driven depletion while fueling $CD8^+$ T cells, thereby improving mitochondrial function and delaying tumor growth [73]. Finally, to balance sustained activity and prevent exhaustion, *Salmonella enterica* was engineered to exploit nonlinear IL-10R expression in the tumor microenvironment. This induced TAMs to produce IL-10, which constrains inflammation while revitalizing exhausted-like $CD8^+$ T cells, thereby achieving durable tumor control [74].

Across these advances, a clear trajectory appears. Bacteria that were initially designed to release immune activators are now being adapted to shape the local immune architecture, bolster metabolic resilience, and fine-tune regulatory balance. Doing so helps them evolve into programmable platforms that can coordinate immune responses with remarkable precision, thereby laying the foundation for versatile therapeutic strategies in complex diseases.

2.3 Drug delivery: Engineered bacteria for targeted *in vivo* transport

Engineered commensals have evolved from simple carriers to multifunctional drug delivery platforms with precise targeting, conditional release, and multimodal therapy. Early work exploited tumor-colonizing EcN equipped with a lysis circuit to release chimeric antigen receptor (CAR) targets anchored to the extracellular matrix, which forms the ProCAR system that enabled antigen-agnostic CAR-T killing and improved infiltration when co-releasing chemokines [75]. Similarly, *Lactobacillus plantarum* WCFS1, naturally binding to nasopharyngeal carcinoma cells via OppA, was engineered to display streptavidin, which loads biotinylated prodrugs for SN-38 release, thereby achieving up to 10-fold potency improvement and 67% tumor inhibition in xenografts [76].

Delivery has progressed toward dynamic sensing. A sialic acid–responsive *E. coli* MG1655 circuit combined NanR-regulated promoters with HlyE expression, activated by surface-decorated sialidases, which simultaneously removes glyco-checkpoints and induces cytolysis [76]. Lactate-sensing EcN integrated quorum-sensing and coagulase modules, which restrict survival to high-lactate tumor zones, release α-hemolysin, and induce coagulation to prevent leakage [77]. Exogenous triggers have been applied. EcN with a TcI42-based switch enabled ultrasound-induced irreversible gene inversion for checkpoint inhibitor release, which restricts activity to irradiated sites [78]. Beyond tumors, engineered bacteria target other tissues. EcN releasing purine nucleobases in the gut enhanced *Clostridia butyrate* metabolism and mucosal repair [79], whereas a commensal that delivered butyrate blocked pyroptosis via HDAC3 inhibition, thereby protecting against colitis [80]. EcN engineered with tryptophan hydroxylase–tryptophan decarboxylase synthesized serotonin, which modulates motility and the microbiota–gut–brain axis [81]. Proteins have been delivered via OMVs employing a modified T0SS, as in uricase-loaded OMVs that achieved superior urate clearance in hyperuricemia models [82]. Cross-system delivery was demonstrated in the nose-to-brain axis. *L. plantarum* WCFS1, which was engineered to secrete leptin, colonized the olfactory epithelium, thereby releasing payloads transported into the brain, where they reduced food intake, improved glucose metabolism, and limited adiposity compared with recombinant leptin [83].

Overall, engineered bacterial delivery has progressed from targeted localization to conditional activation and multi-organ therapy. Bacteria are advancing beyond "living drug factories" toward precise, multifunctional therapeutic systems by integrating microenvironmental cues, exogenous triggers, and cross-tissue routes.

2.4 Diagnostic sensing: Engineered bacteria for *in vivo* disease monitoring and recording

In addition to therapeutic functions, engineered bacteria are programmed to monitor pathological states within the host, which enables early disease diagnosis, temporal recording, and localized responsive interventions [84–86]. This direction has guided the development of theranostic microbial systems that combine diagnosis and therapy, providing advantages of non-invasiveness, programmability, and sustained output [84,86]. Current efforts primarily involve three strategic avenues: sensing pathogenic and inflammatory signals, constructing physiological state–recording circuits, and designing multimodal intelligent interfaces [84,87].

A first line of exploration has focused on programming bacteria as sentinels that detect pathogens or inflammatory cues and immediately translate these signals into diagnostic or therapeutic actions [88]. A notable example is the design of an engineered *Lactococcus lactis* strain that detects quorum-sensing autoinducers

from *Vibrio cholerae*. The strain selectively responded to cholera-specific signals in the intestinal environment by incorporating a hybrid two-component receptor that fuses the cholera autoinducer-1 (CAI-1)–binding domain of CqsS with the signal transduction domain of NisK. Upon detection, the engineered circuit activated a reporter module, which enables pathogen-specific diagnostic readouts in fecal samples while simultaneously conferring colonization resistance against *V. cholerae*. This "sense-and-respond" design demonstrates the feasibility of coupling pathogen detection with real-time intervention for infectious disease treatment [89].

Moreover, engineered bacteria have been designed to act as disposable biosensors or therapeutic carriers by combining inflammation sensing with programmed autolysis. For example, a thiosulfate-responsive *E. coli* circuit was constructed to diagnose and intervene in IBD. The system triggered a dual output that comprised real-time superfolder green fluorescent protein (sfGFP) fluorescence and CRISPR-/Cas9-mediated single-base editing, which records the inflammatory event, upon detecting thiosulfate—a biomarker of intestinal inflammation. Subsequent xylose induction activated lysis E expression, leading to bacterial self-destruction and simultaneous release of the anti-inflammatory protein AvCystatin, thereby enabling safe, one-time use of engineered strains *in situ* [90]. Similarly, diagnostic strategies have been extended to tumor contexts. A recent study used the natural competence of *Acinetobacter baylyi* to detect tumor-derived extracellular DNA using a CRISPR-discriminated horizontal gene transfer (CATCH) assay. Sensor strains integrated oncogenic KRASG12D sequences shed by colorectal tumors, which achieve *in situ* visualization and discrimination of hotspot mutations in organoid models and orthotopic mouse models [88].

Such "sense-and-respond" systems provide real-time intervention, whereas other designs have emphasized temporal depth, thereby enabling bacteria to function as living recorders that archive physiological states and reconstruct disease trajectories over time. Schmidt et al. established the Record-seq platform—which enables transcriptional recording by CRISPR spacer acquisition from RNA—by integrating recording promoters with CRISPR-based transcriptional memory modules. *In vivo* experiments revealed that sentinel *E. coli* cells, which stably colonize the murine gut, could continuously acquire transcriptome-scale information, thereby converting transient RNA expression into durable CRISPR array archives. This system permitted noninvasive monitoring of dynamic host–microbe interactions, including changes in gut permeability and temporal trajectories of inflammatory signals, with data retrievable via fecal sampling and deep sequencing [91]. Further, engineered bacteria are designed as diagnostic probiotics that respond to inflammatory biomarkers. Such strains in a mouse model of IBD achieved related functions of inflammation detection, molecular recording, and personalized therapy, demonstrating the feasibility of a "diagnosis–record–treatment" closed-loop system [92]. Related work further provided a standardized workflow to rapidly develop the i-ROBOT platform for pathological signal archiving and transcriptional recording [93].

Beyond molecular sensing and transcriptional memory, efforts have increasingly moved toward integrating biological circuits with electronic and optical modules, which extend microbial diagnostics into multimodal intelligent interfaces with input and output capacities. Engineered bacterial sensors in early designs primarily relied on single chemical inducers or fluorescent markers. These approaches enabled specific biomarker detection under controlled laboratory conditions; however, their diagnostic performance *in vivo* was frequently limited by background interference and signal instability, thereby hindering their translation into clinical applications. To overcome these limitations, researchers have progressively integrated electronic, optical, and magnetic components, which extend biological sensing into multimodal signal processing. For example, the development of sub-1.4 cm^3 optoelectronic capsules enables real-time, non-invasive readout of inflammation-associated molecular signals from the gastrointestinal tract, which substantially reduces procedural risks [94]. However, most of these systems remain unidirectional, focusing solely on biomarker detection without enabling direct microbial function regulation. Recent advances have achieved a key breakthrough by coupling optogenetically engineered EcN with ingestible optoelectronic capsules, thereby establishing a bidirectional "biological–optical–electronic" communication chain [95]. Within this framework, the engineered microbes sense nitric oxide, an inflammation-associated biomarker, and emit bioluminescent signals that are transduced into electrical signals by the capsule and wirelessly transmitted to a smartphone for diagnosis. In contrast, smartphone commands activate the capsule's light-emitting diodes (LED) module, which optogenetically triggers the engineered bacteria to secrete anti-inflammatory nanobodies, thereby enabling simultaneous early diagnosis and precise therapeutic intervention in a single platform. This evolution represents a conceptual transition in engineered bacterial applications, from being passive therapeutic tools to functioning as dynamic information-processing nodes within the host. Engineered microbes are increasingly positioned as integral components of digital health care systems by operating in a closed-loop cycle of "monitoring–recording–feedback–intervention". With continued refinement of multimodal sensing and integration of multidimensional signal processing, future platforms are expected to support personalized and real-time disease management.

3 External control of engineered bacteria: Precise activation via light, ultrasound, and sensory systems

The expression of functional molecules by engineered bacteria *in vivo* frequently suffers from limited temporal controllability and high background expression, leading to adverse effects or a shift in therapeutic windows [78]. Thus, external control strategies have been explored as complementary approaches to intrinsic genetic designs, which provide an additional regulatory layer to improve precision, responsiveness, and biosafety. To address these challenges, recent studies have introduced external activation strategies, including light and ultrasound, which enable precise spatiotemporal control of drug release and functional expression [78,96]. This approach transforms engineered bacteria from "self-driven" systems into "stimulus-responsive" platforms (Figure 2).

Light signals provide unique advantages as external triggers, being noninvasive, spatially focusable, and characterized by high temporal resolution. Consequently, they have become the most extensively applied input signal in bacterial control systems [97]. Early studies used blue-light–responsive elements to construct optogenetic circuits for reversible regulation of gene expression, which achieve precise control over signal protein or metabolic enzyme production [96]. To accommodate deep-tissue applications, an upconversion micro-nanosystem was developed to convert NIR

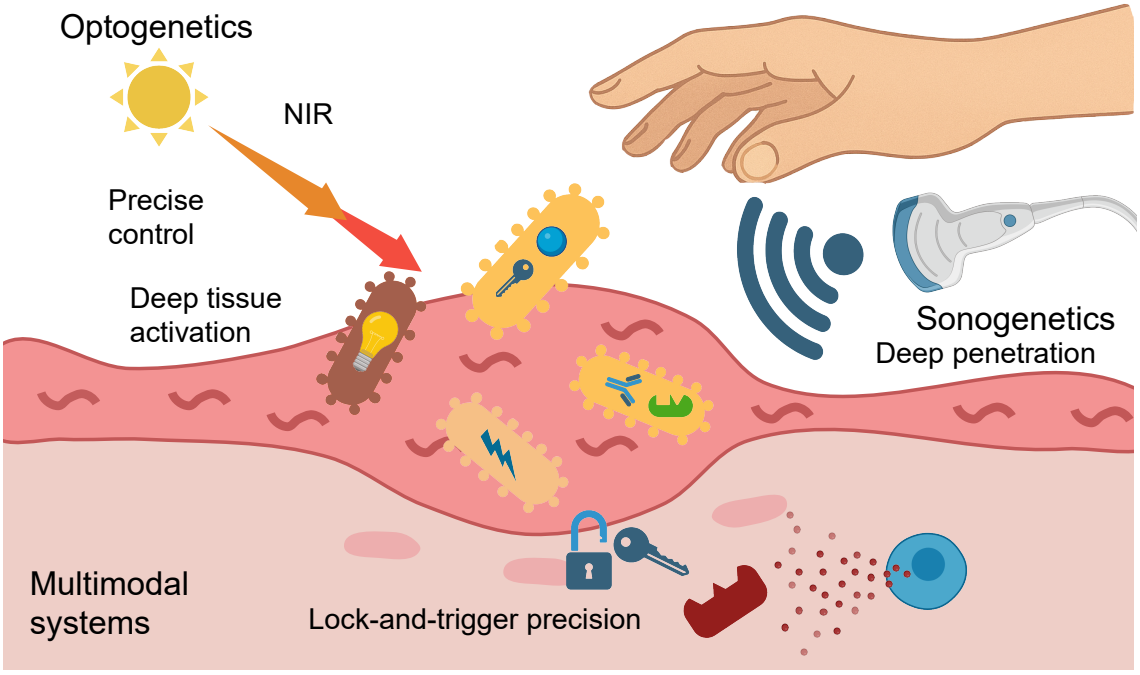

Figure 2 External control of engineered bacteria through optogenetic, sonogenetic, and multimodal systems. Created in https://BioRender.com

light into blue light, thereby enabling optogenetic activation within internal organs [98]. Further optimization integrated engineered bacteria directly into NIR-responsive frameworks, thereby achieving targeted activation of anticancer gene expression within tumor tissues. In this system, localized illumination precisely induced the release of tumoricidal factors at the irradiation site, which balances therapeutic efficacy with tissue safety [99].

Optical approaches, while being powerful, are limited by tissue penetration, which has motivated the development of ultrasound-based systems that provide deep-tissue accessibility and precise spatiotemporal control. Characterized by its deep tissue penetration, high spatial resolution, and tunable frequency, ultrasound has emerged as a powerful modality for controlling engineered bacteria *in vivo*. One representative study established a focused ultrasound–controllable immunotherapy system in which engineered bacteria were programmed with a temperature-actuated genetic switch to release immune checkpoint inhibitors upon local acoustic stimulation. This strategy enabled spatially confined therapeutic activation within solid tumors, thereby markedly improving T cell infiltration and antitumor immune responses in preclinical cancer models [78]. Beyond genetic control, ultrasound served as a physical manipulation tool by exploiting acoustic radiation forces. Recent advances in acoustic tweezers technology demonstrated that gas vesicle-expressing *E. coli* could be trapped, clustered, and programmatically guided *in vivo* using electronically steered ultrasound beams. This approach markedly increased the enrichment of engineered bacteria within tumor sites and potentiated localized therapeutic efficacy [100].

Building upon single-modality strategies, researchers have advanced multimodal control frameworks that integrate optical, acoustic, and endogenous pathological signals. For instance, engineered bacteria can be programmed to enter a "standby state" under specific microenvironmental cues and subsequently release therapeutic payloads only upon external light or ultrasound stimulation, thereby establishing a "lock-and-trigger" mechanism that minimizes systemic toxicity while improving therapeutic selectivity [76]. Such multimodal and synergistic control strategies represent a crucial extension of engineered bacterial design principles, which bridge intrinsic gene circuit regulation with extrinsic environmental responsiveness. They markedly improve the precision and safety of engineered bacterial therapies, thereby serving as a key enabler for future clinical translation and adaptive therapeutic control.

4 Synthetic microbial communities: Advances in system construction and ecological optimization for medical interventions

4.1 Design and construction strategies for synthetic microbial communities

Compared with engineered bacteria, synthetic microbial communities emphasize cooperative functionality, ecological stability, and resilience to perturbation, thereby integrating diverse modules for improved therapeutic outcomes (Table 1). The design concept of synthetic microbial communities originates from attempts to engineer microbial ecosystems. Current strategies are primarily categorized into bottom–up rational design and top–down optimization of natural communities [101]. Researchers frequently start from functional objectives, selecting or constructing key strains through metabolic modeling, functional screening, and genetic engineering, in the bottom–up approach [102]. For example, to improve the production efficiency of short-chain fatty acids, including butyrate, one study introduced co-metabolic pathways and directed interactions, which achieve system-level accumulation of butyrate [103]. Another study revealed that a two-strain consortium provided superior benefits in inflammation modulation and mucosal repair compared with single-strain treatment in a colitis model [103]. More complex designs include constructing engineered communities that target *Fusobacterium nucleatum*–associated colorectal cancer, which effectively regulated

Table 1 Conceptual and functional distinctions between engineered bacteria and synthetic microbial communities in therapeutic design and translation.

Feature	Engineered bacteria	Synthetic microbial communities
Design principle	Genetic circuit modification and modular engineering of a single strain	Assembly of multiple strains based on metabolic complementarity and ecological design
Functional control	Precise, programmable control through internal sensors or external triggers	Emergent collective behavior driven by interspecies interaction
Ecological adaptation	Sensitive to host and environmental perturbations	Enhanced robustness and resilience against ecological disturbances
Colonization and persistence	Typically transient or controllable clearance	Designed for long-term stability and niche occupation
Mechanistic transparency	Mechanisms are clearly defined and experimentally traceable	Complex emergent properties requiring system-level analysis
Scalability and optimization	New functions added via iterative circuit design	Adding or removing strains requires ecological network rebalancing
Manufacturing and GMP	Standardized single-strain fermentation	More challenging batch consistency and composition control
Dosing and administration	Dose–response relationships are well defined	Requires coordinated dosing, inoculation order, and ratio management
Monitoring and biomarkers	Easily tracked via circuit-specific markers	Requires multi-omics or metabolic profiling for system-level readout
Risks and failure modes	Overexpression, leakage, or immunogenicity	Ecological drift, interspecies competition, or functional loss
Regulatory focus	Clear pathway for single-strain live biotherapeutic products	Complex quality and risk evaluation for multistrain consortia
Clinical suitability	Ideal for acute, localized, and switchable interventions	Promising for chronic, multifactorial, and microbiota-related disorders

its ecological niche, suppressed pathogen growth, and improved the host metabolic status [104].

Top–down strategies emphasize functional compression and ecological optimization of natural microbial communities. Researchers have conducted strain-deletion experiments to identify key metabolic drivers [105,106] and further applied metabolic modeling and community interaction networks to identify core components of stability. Hence, targeted genome editing or synthetic ecological module assembly can be used to reconstruct synthetic consortia with specific ecological functions. For example, *in situ* base editing of defined strains has been achieved in the murine gut [107]. Further, a synthetic microbial community consisting of seven commensal species has been constructed to improve the ecological barrier and suppress vancomycin-resistant bacteria [108]. Such strategies integrate functionality, ecological stability, and controllability and represent an important direction in current research on synthetic microbial communities (Figure 1). However, despite these advances, maintaining long-term ecological stability remains challenging as metabolic trade-offs and interspecies competition can destabilize designed consortia over time [109]. Recent work further indicates that minimizing resource overlap and incorporating narrow-spectrum resource–utilizing strains enhances cooperative potential and community stability [110].

A practical contrast between bottom–up and top–down paradigms is emerging. Bottom–up designs prioritize functional specificity and mechanistic control, thereby enabling rational tuning of cross-feeding, flux partitioning, and signaling through defined genetic/interaction modules. However, higher-order interactions and context-dependent effects reduce predictability and robustness when moved from simplified settings to complex hosts as the community size grows [111]. By comparison, top–down reconstruction compresses natural communities to retain pre-adapted interaction networks that confer colonization resistance and ecological resilience *in vivo*; however, this frequently comes with lower design flexibility and less precise control over per-strain outputs [112,113]. Moreover, interindividual differences in the human microbiome impose colonization barriers and competitive exclusion; thus, communities that are stable in one host may not transfer predictably to another—an issue that bottom–up consortia are especially sensitive to [114]. Therefore, a hybrid route is attractive. A top–down, stability-anchored scaffold (ecologically coherent and colonization-competent) was used, and bottom–up engineered strains that deliver well-specified functions are embedded. Iterative modeling/measurement then reconciles control with resilience, thereby improving portability from gnotobiotic models to human-relevant contexts [111,113]. Collectively, this integrated perspective identified when to favor precision (bottom–up) versus robustness (top–down) and why hybrid designs are promising for predictable, translational SynComs.

4.2 Colonization efficiency and ecological stability

The clinical application of synthetic microbial consortia critically relies on their colonization efficiency and ecological stability within the host intestine. Multiple animal models and preclinical studies have demonstrated the potential of microbiota interventions; however, colonization outcomes remain highly heterogeneous across individuals. A study reported the close association of probiotic colonization with the composition of the native microbiota, mucosal immune status, and secretory IgA levels [115]. Another study indicated that most probiotics fail to persist long term within the host gut under conventional oral administration [116].

The following strategies have been developed to enhance colonization efficiency: (1) engineering strains to express targeted adhesion factors that increase epithelial specificity; (2) designing "co-inoculation" strategies that introduce colonization-promoting microbes or community-structuring enhancers; and (3) applying niche simulators or substrate supplements to selectively expand

desired populations. Further, delivery technologies, including nanoencapsulation and lyophilization, have been investigated to improve the oral stability of probiotics, thereby providing critical support for clinical formulations. However, stochastic colonization dynamics, host variability, and competition with native microbiota frequently lead to unpredictable outcomes and ecological drift, highlighting the challenge of achieving stable coexistence in complex environments [110,117].

4.3 Applications and preclinical studies: From functional validation to model-based interventions

The application of synthetic microbial communities has expanded to diverse disease contexts, including colitis, infections, metabolic disorders, and even neuropsychiatric conditions. In bottom–up approaches, a two-strain consortium for chronic immune-mediated colitis [103] was demonstrated in mouse models to restore intestinal homeostasis and considerably reduce inflammation. Other work on butyrate-producing optimized consortia revealed symptom relief and colonic barrier integrity enhancement in models of IBS. Engineered consortia have been designed for cancer-related interventions to remodel the colorectal tumor microenvironment, interfere with pathogen colonization, or induce antitumor immunity [104].

In contrast, top–down applications place greater emphasis on reshaping community structures and stripping functions to their crucial components. Systematic strain-deletion studies have revealed key nodes of cooperative metabolism [105,118], which were subsequently utilized to design minimal but functional consortia. Among disease targets, *Clostridioides difficile* infection has become one of the most actively investigated areas, with multiple animal studies validating the efficacy of rationally designed synthetic communities [119,120]. In the context of antimicrobial resistance, a seven-member synthetic community effectively reinforced the endogenous microbiota barrier and restricted the spread of vancomycin-resistant enterococci, thereby providing a promising prophylactic strategy for hospitalized patients [108]. Despite these encouraging results, most synthetic communities have only been assessed in short-term animal studies, and their ecological resilience, long-term coexistence, and translational reproducibility remain major challenges for clinical application [121].

5 Clinical risk assessment, management, and future directions

Engineered bacteria and synthetic consortia provide therapeutic promise; however, clinical translation requires balancing efficacy with risks that involve biosafety, ethics, regulation, and technical limits. Risk assessment and management are crucial, whereas emerging technologies will drive precision medicine. This section outlines biosafety and containment, ethical implications, regulatory frameworks, and future perspectives.

Several intrinsic technological limitations remain despite rapid progress. First, CRISPR-based editing and regulation still produce off-target and context-dependent effects, raising safety and durability concerns for engineered live systems [122,123]. Second, immunogenicity may constrain repeated or sustained use. Pre-existing humoral and T-cell responses to extensively used Cas9 orthologs have been documented in humans, implying dosing, persistence, and monitoring [124]. Third, host responses and dose-related toxicities limit efficacy despite encouraging preclinical data when bacterial vectors are deployed in humans, as illustrated by the attenuated *Salmonella* VNP20009 phase-I experience [125]. Finally, ethical and governance considerations—particularly around permanent genetic modifications and environmental dissemination—highlight the need for reversibility, containment, and proportionate oversight in clinical translation [126]. Addressing these constraints through safer editing architectures, immuno-aware design, and robust biocontainment will be crucial for responsible advancement [122–126].

These intrinsic technological and ethical constraints form the foundation of broader biosafety concerns, which become more pronounced during clinical implementation and patient exposure. Systemic risks span infection, immune responses, horizontal gene transfer (HGT), and ecological perturbations. Infection risk appears when genetic modifications improve survival, thereby causing opportunistic infections or sepsis in immunocompromised patients [127]. Trials have reported gastrointestinal discomfort or bacteremia, particularly at high doses [128]. Further, pathogen transmission has occurred in FMT-based therapies [129]. Immune responses to exogenous proteins or metabolites may trigger allergies or autoimmunity; whereas, inflammation benefits tumor therapy but may escalate to cytokine storms or intestinal damage [130]. Synthetic consortia may disrupt native microbiota, causing dysbiosis and aggravating autoimmune, neurodegenerative, or neoplastic diseases [130]. As plasmids may disseminate antibiotic resistance or functional modules to environmental microbes, HGT remains critical [130]. Comprehensive assessment requires *in vitro* models, animal studies, and human cohorts to identify high-risk populations and scenarios [131].

Beyond biosafety, a key translational barrier lies in bridging from animal models to human settings. Several promising microbial treatments that succeed in murine systems fail to replicate effects in human hosts due to interspecies differences in immunity, microbiome ecology, and host–microbe interactions [132,133]. Animal models frequently do not capture complex human-associated microbial functions or organ-level interactions, thereby limiting their predictive power [134]. Moreover, regulatory frameworks for live biotherapeutic products (LBPs) impose strict criteria on manufacturing consistency, strain stability, and biosafety, which are often underdeveloped in early-stage microbial therapeutics [135–137]. Bridging these translational gaps will require humanized models, organ-on-chip systems, longitudinal microbiome monitoring, and iterative regulatory engagement.

Mitigation strategies include biocontainment, regulatory control, and clinical monitoring. Kill switches, such as nutrient dependency or environment-responsive circuits, enable engineered strains to self-eliminate outside target sites [127]. The “Deadman” system lyses cells upon external cues [138]. CRISPR–Cas9 enhances stability and reduces HGT probability [139]. Hydrogel encapsulation further improves containment [128]. Regulatory oversight is stringent, with the United States Food and Drug Administration (FDA) classifying engineered strains as “LBPs”, thereby requiring environmental risk assessments and safety validation [131]. Preclinical toxicology mandates dose-escalation and immunogenicity tests [127]. Clinical monitoring uses metagenomic sequencing and biomarkers to track colonization and adverse events. These strategies have supported the progression of several therapeutics into phase II/III trials [129]. Regulatory and manufacturing frameworks are pivotal for LBP translation. In the United States, the FDA classifies engineered

microbial therapeutics as LBPs and specifies the Chemistry, Manufacturing, and Controls information required for Investigational New Drug Application submissions, including strain identity, genetic stability, process controls, release criteria, and environmental risk considerations [140]. In Europe, evolving European Medicines Agency/European Union discussions emphasize the need for a defined framework tailored to viable microbial products, particularly focusing on risk analysis, consistency, and lifecycle control [135]. From a GMP standpoint, LBPs face unique challenges—including maintaining viability and potency, minimizing batch-to-batch variability, and establishing robust analytics for complex, living products—which are now increasingly addressed through fit-for-purpose manufacturing and characterization strategies [141]. Real-world experiences replicate these constraints, with SYNB1618/SYNB1934 progressing through early- and mid-stage evaluation under these requirements [8,142,143]. Meanwhile, the attenuated Salmonella VNP20009 exhibited limited clinical activity and dose-related toxicities despite tumor colonization in a phase I study [125]. Collectively, these cases underscore the integration of synthetic-biology design with regulatory expectations and GMP controls to ensure reproducibility, scalability, and patient safety.

Future applications will emphasize precision, intelligence, and multimodality. Personalized treatment may combine artificial intelligence (AI)–driven microbiome modeling with host genotyping for tailored microbial designs [144]. Emerging methods, such as nanoencapsulation and optoacoustic control, will improve spatiotemporal precision, thereby enabling on-demand activation and reduced toxicity [127]. Cross-disciplinary integration, including synergy with CAR-T or nanomedicines, will accelerate translation [145]. Microbial therapeutics are poised to enter clinical practice as sustainable and minimally invasive interventions for chronic disease as multicenter trials expand and regulation matures [146].

6 Conclusion

The rapid progress of synthetic biology has transformed engineered bacteria and synthetic microbial communities from experimental concepts into promising therapeutic platforms. These systems are evolving into programmable "living drugs" that address complex and chronic diseases by integrating modules for metabolic compensation, immune activation, drug delivery, diagnostic sensing, and external control. Preclinical and early clinical studies have already demonstrated encouraging outcomes in metabolic disorders, cancer immunotherapy, and gut–brain axis regulation, emphasizing the feasibility of microbial therapeutics as next-generation interventions.

However, challenges in ensuring biosafety, colonization stability, and precise spatiotemporal regulation *in vivo* remain. The risk of HGT, ecological perturbations, and immune overactivation must be carefully addressed via biocontainment strategies, standardized manufacturing, and rigorous clinical evaluation. Importantly, future advances will depend on interdisciplinary integration. The convergence of microbiology, synthetic biology, systems biology, and AI will accelerate the design of intelligent and personalized microbial therapies.

Looking forward, engineered bacteria and synthetic microbial consortia are expected to shift from empirical administration to precision therapeutics, thereby ultimately providing sustainable, safe, and minimally invasive options for human health. With continued technological refinement and clinical validation, microbial therapeutics play a pivotal role in the future of precision medicine (Figure 1).

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Author contribution statement

Fangyuan Ye performed detailed literature collection and analysis, drafted the manuscript sections, organized reference materials, and refined the academic expressions. Hao Zheng conceived the review framework, determined the thematic focus, coordinated interdisciplinary perspectives, and oversaw the overall academic quality. All authors have approved the final manuscript.

Consent

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Data availability

Not applicable.

Declaration of competing interest

Both contributing authors report no conflicts of interest in this work.

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Use of AI statement

None.

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