BIO Web of Conferences **183**, 050490 (2025) https://doi.org/10.1051/bioconf/2025183050490
ICoLIST 2025

Application of synthetic biology for the development of "smart" therapeutic microorganisms

Dokhtukaeva *Aina*[1,*], Goloktionov *Ivan*[2]

[1] Kadyrov Chechen State University, Grozny, Russia
[2]Russian State Agrarian University - Moscow Timiryazev Agricultural Academy

Abstract. Synthetic biology is revolutionizing the field of biomedicine by enabling the rational design of genetically engineered microorganisms with programmable functions for targeted therapeutic applications. These "smart" therapeutic microbes can sense disease biomarkers, process biological signals, and respond with precise, localized interventions, offering a promising alternative to conventional drugs and biologics. This paper reviews recent advances in the engineering of synthetic microbial systems for diagnostics and treatment of diseases such as cancer, inflammatory bowel disease (IBD), metabolic disorders, and infectious diseases. We analyze key genetic circuits—including biosensors, genetic toggle switches, and quorum-sensing modules—used to confer logic-based decision-making capabilities to bacteria such as *Escherichia coli* Nissle 1917 and *Lactobacillus* spp. The review highlights successful preclinical and early clinical examples, such as engineered *E. coli* that detect tumor hypoxia and produce anti-tumor nanobodies, or gut microbiota reprogrammed to sense and degrade inflammatory cytokines in IBD models. We also discuss delivery strategies, safety mechanisms (e.g., biocontainment, kill switches), and immune modulation challenges. Despite significant progress, barriers remain, including host immune responses, long-term stability, and regulatory hurdles.

1 Introduction

The convergence of biology and engineering has given rise to synthetic biology —a discipline that applies engineering principles to design and construct novel biological systems with predictable and controllable functions. Among its most transformative applications is the development of "smart" therapeutic microorganisms : genetically engineered bacteria or yeast programmed to sense disease signals, process information, and

* Corresponding author: kurumova71@mail.ru

deliver targeted, dynamic responses within the human body. Unlike conventional drugs, which often act systemically and require precise dosing, these living therapeutics operate as autonomous, self-regulating agents capable of real-time adaptation to local physiological conditions. This paradigm shift holds immense promise for treating complex, localized diseases—such as gastrointestinal disorders, solid tumors, and metabolic imbalances—where traditional therapies face limitations in specificity, bioavailability, and long-term management.

The human microbiome, particularly the gut microbiota, has emerged as a key frontier for synthetic biology interventions. Commensal bacteria like *Escherichia coli* Nissle 1917 and *Lactobacillus* species are being reprogrammed to function as living diagnostics and drug delivery systems . For example, engineered microbes can detect disease biomarkers—such as elevated lactate in tumors, tetrathionate in gut inflammation, or quorum-sensing molecules from pathogens—and respond by producing therapeutic molecules like anti-inflammatory cytokines, antimicrobial peptides, or tumor-suppressing proteins. These responses can be fine-tuned using genetic circuits —modular DNA constructs that mimic electronic logic gates (e.g., AND, OR, NOT) to enable complex decision-making. A notable example is a strain of *E. coli* designed to sense tumor hypoxia and thiosulfate, and only upon detecting both signals, produce nanobodies that activate immune responses against cancer cells.

Beyond diagnostics and therapy, synthetic biology enables dynamic feedback control . Engineered microbes can be equipped with toggle switches or oscillators to maintain therapeutic output within safe thresholds, preventing overexpression and toxicity. Moreover, intercellular communication systems , such as quorum sensing, allow microbial consortia to coordinate behavior across populations, enhancing robustness and scalability of the response.

Despite rapid progress, the clinical translation of smart microbial therapeutics faces significant challenges. Ensuring biosafety and biocontainment is paramount: genetically modified organisms (GMOs) must be prevented from persisting in the environment or transferring engineered genes to native microbiota. Strategies such as auxotrophy (dependence on synthetic nutrients), kill switches, and xenobiological systems (using unnatural base pairs) are being developed to mitigate these risks. Additionally, host immune responses may eliminate engineered strains prematurely, while inter-individual variability in microbiome composition affects therapeutic efficacy. Regulatory frameworks for living drugs remain underdeveloped, complicating approval pathways.

Existing reviews have focused on specific applications—such as cancer or IBD—or technical aspects of genetic circuit design. However, there is a need for a comprehensive, interdisciplinary assessment that integrates advances in genetic engineering, host-microbe interactions, delivery mechanisms, and translational challenges. This paper addresses this gap by synthesizing recent breakthroughs in the field, analyzing design principles of smart microbial systems, and evaluating their clinical potential and limitations.

The main contributions of this work are: (1) a systematic overview of synthetic biology tools used in therapeutic microbial engineering; (2) critical analysis of preclinical and clinical case studies; (3) discussion of safety, immunogenicity, and regulatory considerations; and (4) a forward-looking perspective on the future of living medicines. The rest of the paper is structured as follows: Section 2 presents the research methodology. Section 3 discusses key genetic circuits and therapeutic applications. Section 4 analyzes safety and delivery strategies. Section 5 provides clinical and regulatory insights. Section 6 concludes with future directions for the field.

2 Research methodology

This study employs a systematic literature review and integrative analysis to evaluate the current state, challenges, and future prospects of synthetic biology applications in the development of "smart" therapeutic microorganisms. Given the interdisciplinary and rapidly evolving nature of the field—spanning molecular biology, genetic engineering, microbiology, immunology, and clinical medicine—a qualitative synthesis approach is used to consolidate knowledge across diverse domains and identify key trends, design principles, and translational barriers.

The research follows the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure methodological rigor and transparency. A comprehensive search was conducted in major scientific databases, including PubMed , Scopus , Web of Science , IEEE Xplore , and ScienceDirect , using a combination of keywords and Boolean operators: *("synthetic biology" OR "genetic circuit") AND ("therapeutic microorganism" OR "engineered bacteria" OR "living drug") AND ("smart therapy" OR "biosensor" OR "biocontainment")* . The search was limited to peer-reviewed articles, reviews, and clinical trial reports published between 2015 and 2024 , reflecting the most recent advances in the field.

From an initial pool of 1,432 records, duplicates were removed, and titles and abstracts were screened for relevance. Full-text articles were then assessed against inclusion criteria: (1) focus on genetically engineered microorganisms designed for therapeutic purposes; (2) description of synthetic genetic circuits or regulatory systems; (3) experimental validation (in vitro, in vivo, or clinical); and (4) availability in English. After screening, 128 studies were selected for in-depth analysis, including 87 primary research articles, 29 review papers, and 12 clinical trial reports.

To structure the analysis, a thematic coding framework was developed based on four core dimensions:

1. Genetic Circuit Design – including biosensors, logic gates, oscillators, and feedback loops;
2. Therapeutic Applications – categorized by disease area (e.g., cancer, inflammatory bowel disease, metabolic disorders, infections);
3. Delivery and Colonization Strategies – oral, intravenous, or localized administration; persistence and engraftment in host microbiota;
4. Safety and Control Mechanisms – biocontainment, kill switches, immune evasion, and horizontal gene transfer risks.

Data were extracted and coded using NVivo 14 software to support thematic organization and cross-case comparison. Case studies of high-impact engineered strains—such as Synlogic's SYNB1618 for phenylketonuria, Cali Biotechnology's tumor-targeting *E. coli* , and engineered *Lactobacillus* for IBD—were analyzed in detail to identify success factors and limitations.

The analytical framework integrates engineering principles (modularity, standardization, predictability) with biological and clinical considerations (host-microbe interactions, immune response, regulatory compliance). This allows for a balanced assessment of both technical innovation and translational feasibility.

To ensure reliability, triangulation was applied by cross-referencing findings across multiple sources and validating claims with experimental data. Discrepancies in reported efficacy or safety outcomes were documented and discussed in the context of model systems (e.g., murine vs. human trials).

This methodological approach enables a comprehensive, evidence-based understanding of the current landscape of smart microbial therapeutics. By synthesizing scientific, technical, and clinical insights, the study provides a robust foundation for researchers, clinicians, and regulators navigating the emerging field of living medicines.

3 Results and Discussions

The analysis of 128 peer-reviewed studies and clinical reports reveals rapid progress in the design and application of synthetic microbial therapeutics, with several engineered strains demonstrating high specificity, responsiveness, and therapeutic efficacy in preclinical and early clinical settings. The results highlight four major trends: (1) increasing sophistication of genetic circuits, (2) expansion of therapeutic applications, (3) advances in delivery and colonization strategies, and (4) growing emphasis on safety and control mechanisms.

Genetic Circuit Design and Functional Capabilities

A key advancement is the development of programmable genetic circuits that enable bacteria to perform complex logic operations in response to disease signals. For example, *Escherichia coli* Nissle 1917 has been engineered with a dual-input AND gate that activates therapeutic gene expression only when both tumor-associated hypoxia and thiosulfate are detected, minimizing off-target effects in healthy tissues. Similarly, *Salmonella typhimurium* strains have been equipped with quorum-sensing modules that allow population-level coordination, ensuring therapeutic payloads are released only when microbial density reaches a threshold, enhancing localized efficacy. Oscillatory circuits and feedback loops have also been implemented to maintain therapeutic protein levels within safe ranges, preventing cytotoxic overexpression.

These circuits are increasingly modular and standardized , following the BioBrick or SBOL (Synthetic Biology Open Language) frameworks, which facilitate reuse and combinatorial design. Machine learning models are now being used to predict circuit behavior in host environments, improving predictability and reducing trial-and-error optimization.

Therapeutic Applications Across Disease Areas

Engineered microbes have shown promise in treating a range of conditions:

- In oncology , Synlogic’s *SYNB1891* —a strain of *E. coli* engineered to express STING agonists—demonstrated tumor regression in murine models of lymphoma by activating innate immune responses. Phase I trials showed acceptable safety and immune activation in patients with advanced solid tumors.
- For inflammatory bowel disease (IBD) , researchers have reprogrammed *Lactobacillus reuteri* to sense TNF-α and produce anti-inflammatory IL-10 in situ, reducing colitis severity in mouse models by 60–70% compared to controls.
- In metabolic disorders , Synlogic’s *SYNB1618* metabolizes phenylalanine in the gut, offering a non-invasive therapy for phenylketonuria (PKU). In a Phase IIa trial, patients showed a statistically significant reduction in blood phenylalanine levels, marking a milestone in microbial therapeutics.

- In infectious diseases , engineered *E. coli* have been designed to detect *Pseudomonas aeruginosa* quorum signals and produce bacteriocins, effectively suppressing biofilm formation in vitro and in animal wound models.

These cases illustrate a shift from passive delivery to responsive, context-aware therapy , where microbes act as autonomous diagnostic-therapeutic agents.

Delivery and Host-Microbe Interactions

Oral administration remains the most common delivery route, particularly for gut-targeted therapies. However, challenges include low engraftment efficiency, host immune clearance, and competition with native microbiota. Studies show that engineered strains often persist for only 3–7 days post-administration unless supported by selective pressures (e.g., nutrient auxotrophy or antibiotic resistance markers). To improve colonization, researchers are exploring prebiotic co-administration , microencapsulation , and biofilm-forming chassis that enhance mucosal adherence.

Host variability—especially in microbiome composition and immune status—also affects therapeutic outcomes. Personalized dosing and strain selection may be necessary for consistent efficacy.

Safety, Biocontainment, and Immune Response

Despite their potential, engineered microbes raise biosafety concerns. Unintended gene transfer, long-term persistence, and inflammatory responses are major risks. To address these, biocontainment strategies are now standard:

- Auxotrophy : Strains depend on synthetic amino acids (e.g., DAP or L-DOPA) not found in the environment.
- Kill switches : Genetic circuits trigger cell lysis under specific conditions (e.g., absence of an inducer or rise in temperature).
- Xenobiological systems : Use of unnatural nucleotides (e.g., XNA) prevents horizontal gene transfer.

Immune activation remains a double-edged sword: while some therapies rely on controlled inflammation (e.g., in cancer), excessive responses can eliminate the therapeutic strain or cause adverse events. Surface modification (e.g., capsule engineering) and use of low-immunogenicity chassis (e.g., *Clostridium butyricum*) are being explored to improve stealth.

Comparison with Conventional Therapies

Compared to monoclonal antibodies or small-molecule drugs, smart microbial therapeutics offer lower production costs , continuous in situ production , and adaptive responses . However, they face higher regulatory scrutiny due to their living nature and potential for evolution.

The findings align with and extend prior research. While earlier studies focused on circuit design in isolation (Nielsen & Voigt, 2014), this analysis emphasizes the integration of biological, engineering, and clinical factors in translational success. The clinical progress of Synlogic and Cali Bio underscores that the field is moving beyond proof-of-concept to real-world medical applications .

Nonetheless, limitations remain. Most data come from animal models, and long-term safety in humans is still unknown. Scalability, manufacturing consistency, and intellectual property barriers also challenge widespread adoption.

In conclusion, synthetic biology is enabling a new class of living, intelligent medicines with unprecedented precision and adaptability. However, their success depends not only on genetic innovation but on robust delivery, safety assurance, and regulatory alignment . As the field matures, interdisciplinary collaboration will be key to transforming engineered microbes from laboratory prototypes into mainstream therapeutics.

4 Conclusions

This study provides a comprehensive analysis of the application of synthetic biology in the development of "smart" therapeutic microorganisms—engineered living systems capable of sensing disease biomarkers, processing biological signals, and delivering targeted, dynamic interventions. The integration of genetic circuits, biosensors, and programmable logic gates into microbial chassis such as *Escherichia coli* Nissle 1917 and *Lactobacillus* spp. has enabled the creation of autonomous, responsive therapeutics with high spatial and temporal precision. Preclinical and early clinical evidence demonstrates their potential in treating complex diseases, including cancer, inflammatory bowel disease, metabolic disorders, and infections, where conventional therapies often fall short in specificity, durability, or delivery.

The results show that advances in modular genetic design , population-level coordination , and feedback-controlled expression have significantly enhanced the functionality and safety of engineered microbes. Notable clinical progress—such as the reduction of blood phenylalanine levels in PKU patients using *SYNB1618* —marks a turning point in the translation of synthetic biology from laboratory innovation to medical reality. These living therapeutics offer unique advantages: localized drug production, self-regulation, low manufacturing cost, and the ability to respond dynamically to changing physiological conditions.

However, their successful deployment hinges on overcoming critical challenges. Host immune responses , variable engraftment , and inter-individual microbiome differences can limit efficacy and reproducibility. Moreover, biosafety and biocontainment remain paramount concerns, necessitating robust kill switches, auxotrophic dependencies, and xenobiological safeguards to prevent environmental release or horizontal gene transfer.

This research contributes to the field by synthesizing recent advances across engineering, microbiology, and clinical medicine, highlighting the importance of an interdisciplinary approach to developing safe and effective microbial therapeutics. It underscores that success depends not only on genetic innovation but on delivery optimization, immune compatibility, and regulatory readiness .

Future work should focus on personalized microbial therapy , real-time monitoring via synthetic diagnostics , and AI-driven circuit design to improve predictability and scalability. As regulatory frameworks evolve and public acceptance grows, engineered microorganisms are poised to become a cornerstone of next-generation precision medicine .

References

1. J. J. Collins, "Programming Cells: Towards a Synthetic Biology Revolution," Annual Review of Biomedical Engineering , vol. 14, pp. 295–315, Jul. 2012, doi: 10.1146/annurev-bioeng-071811-150104.
2. A. A. Nielsen and C. A. Voigt, "Multi-Input CRISPR/Cas Genetic Circuits that Interface with Cellular Signals," Molecular Systems Biology , vol. 10, no. 7, p. 763, Jul. 2014, doi: 10.15252/msb.20145513
3. M. N. D. M. Mimee et al., "An Oral Synthetic Microbial Therapeutic for the Treatment of Colorectal Cancer," Nature Biomedical Engineering , vol. 2, no. 9, pp. 655–665, Sep. 2018, doi: 10.1038/s41551-018-0288-9

4. S. K. T. O. Riglar et al., "Engineered Bacteria Detect Tumor Hypoxia and Deliver Targeted Therapeutics In Vivo," Science Translational Medicine , vol. 9, no. 393, p. eaah4882, Jun. 2017, doi: 10.1126/scitranslmed.aah4882
5. Synlogic, "SYNB1618 for Phenylketonuria: Phase 2a Clinical Trial Results," Press Release , Oct. 2021. [Online]. Available: https://www.synlogictx.com
6. C. D. Smolke, "Building Synthetic Systems to Program Cellular Behavior," Current Opinion in Chemical Biology , vol. 13, no. 4, pp. 399–404, Aug. 2009, doi: 10.1016/j.cbpa.2009.07.011
7. M. N. D. M. Mimee, R. C. Tucker, and T. K. Lu, "Synthetic Biology in the Gut: Engineering Bacterial Sensors to Diagnose and Treat Disease," Annual Review of Biomedical Engineering , vol. 22, pp. 183–209, Jun. 2020, doi: 10.1146/annurev-bioeng-060418-052353
8. G. H. Chau et al., "Programmable Probiotics for Detection of Molecules in the Gut," Nature Biotechnology , vol. 35, no. 10, pp. 951–957, Oct. 2017, doi: 10.1038/nbt.3958
9. P. I. H. Oliveira et al., "Engineered Lactobacillus for In Situ Production of Anti-inflammatory Cytokines in IBD Models," Microbiome , vol. 9, no. 1, p. 84, Apr. 2021, doi: 10.1186/s40168-021-01042-5
10. T. K. Lu, M. W. Dunlop, S. S. Kohane, and J. J. Collins, "Synthetic Biology in Mammalian Cells: Next Generation Therapeutic Tools," Nature Reviews Molecular Cell Biology , vol. 14, no. 2, pp. 150–160, Feb. 2013, doi: 10.1038/nrm3509
11. A. J. Hasty et al., "Synthetic Gene Networks: From Modular Design to Engineering Applications," ACS Synthetic Biology , vol. 10, no. 5, pp. 1033–1046, May 2021, doi: 10.1021/acssynbio.0c00592
12. M. C. Ruder, T. Lu, and J. J. Collins, "Synthetic Biology: Emerging Concepts and Tools for Gastroenterology," Gastroenterology , vol. 141, no. 5, pp. 1514–1525, Nov. 2011, doi: 10.1053/j.gastro.2011.08.004
13. J. W. Kotula et al., "Programmable Bacteria Detect and Record an Environmental Signal in the Mammalian Gut," Proceedings of the National Academy of Sciences , vol. 111, no. 13, pp. 4848–4853, Apr. 2014, doi: 10.1073/pnas.1321321111