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Spotlight

Coordinated microbial lysis bursts into the drug delivery scene

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To address limitations in dosing and releasing cargo from engineered microbes, Din *et al.* harnessed a previously designed oscillatory genetic circuit to achieve the synchronized release of cancer-killing protein payloads. Here, we briefly recap this study published in 2016 and its transformative impact on the field.

Engineered microbes are a promising area of study for therapeutic development. This is because microbes exhibit tropism to regions within the human body, can penetrate difficult to drug microenvironments such as tumor cores, and can be engineered for the biosynthesis of therapeutic molecules within patients, amongst other benefits. The history of microbes in medicine is extensive; microbial therapies have been utilized since the 19th century and are currently under evaluation for treatment of various diseases, including metabolic disorders, cancer, and gastrointestinal disease [1]. Microbes can also be considered as a chassis for non-invasive delivery of small molecule or protein drugs, as the molecules that they manufacture can be generated and delivered within the patient without the need for downstream separation costs that are currently incurred in centralized pharmaceutical manufacturing. Notably, while numerous protein-based therapies have been developed, sometimes the delivery of these therapies to target regions can be challenging, such as in the treatment of cancerous tumors [2]. As such, target-homing microbes could be considered for the delivery of these therapies. However, microbial therapeutics delivering proteinaceous cargo have achieved limited clinical success in part due to insufficient delivery of appropriate dosages through secretory pathways [1]. In 2016, Din and colleagues [3] helped address these challenges by engineering a bacterial strain that enhances local delivery of protein payloads in self-regulating doses. They achieved this through a conceptual advance in genetic circuit design that enabled bacteria to synchronously lyse and deliver biosynthesized cargo.

Genetic circuit research has generated tools to control and augment biological functionality. This paradigm emerged in the early 21st century, when synthetic processes based on the central dogma of biology began to be mathematically modeled and developed akin to logical circuit components. Several foundational works in synthetic biology reported the design of oscillatory networks. Prior work by Danino and coworkers [4] generated a microbial oscillator, where accumulation of quorum-sensing molecule acyl-homoserine lactone (AHL) was used to trigger cyclical gene expression behavior. AHL accumulation within a cell population enables the AHL-LuxR transcriptional complex to initiate expression of both LuxI, which forms AHL, as well as AiiA, which breaks down AHL, providing the positive and negative regulation required for oscillation. This development served as the foundation for a synchronized lysis circuit (SLC) utilized by Din, Danino, and coworkers. For the SLC, positive and negative feedback were implemented through the expression of LuxI and the bacteriophage lysis gene φX17E, respectively. As visualized within a microfluidic device, clusters of cells accumulated, generated critical masses of AHL, and then collectively lysed, releasing their intracellular contents. Further circuit development with an ssrA degron attached to LuxI in the tumor-homing *Salmonella typhimurium* strain led to a genetic circuit with increased lysis threshold and accelerated periodicity. Thus, the SLC enabled cyclical growth and lysis of a bacterial population, a function new to nature.

The ability to improve the timing and release of biosynthesized payloads in microbes could enhance on-target response and limit off-target response. The team harnessed their SLC to release intracellular therapeutic cargo at controlled dosages and simultaneously reset the bacterial population. They prototyped and translated their technology from release of a fluorescent reporter protein in a microfluidic device through to delivery of a therapeutic protein in a mouse. First, to test the delivery of antitumor toxin hemolysin E using the SLC, the authors cocultured HeLa cells and an *S. typhimurium* strain that carried an AHL-LuxR activated *hlyE* gene expression cassette within a microfluidic device. Decreased HeLa viability suggested HeLa cell death upon SLC-mediated lysis, showing the SLC was capable of toxic payload delivery to a human cell culture. The authors moved from *in vitro* to *in vivo* models by evaluating murine subcutaneous tumor colonization of SLC-carrying bacteria. They observed pulsatile growth of SLC-carrying bacteria inside tumors using a luminescent reporter. Subsequently, intratumoral injection of three SLC strains bearing immune response-triggering genes led to significant decreases in relative tumor volume. Their culminating experiment was the screening of an SLC-carrying strain as a therapeutic to treat colorectal metastases within the liver of mice. Treatment in conjunction with chemotherapy drug 5-FU led to an observed 30% reduction in tumor activity and a 50% increase in mean survival time, suggesting a joint impact of tumor-targeting microbes and vasculature-targeting drugs. This finding revealed that synchronized lysis delivery can be used to deliver a drug payload to murine tumors, a milestone in the design of novel therapeutic microbes.

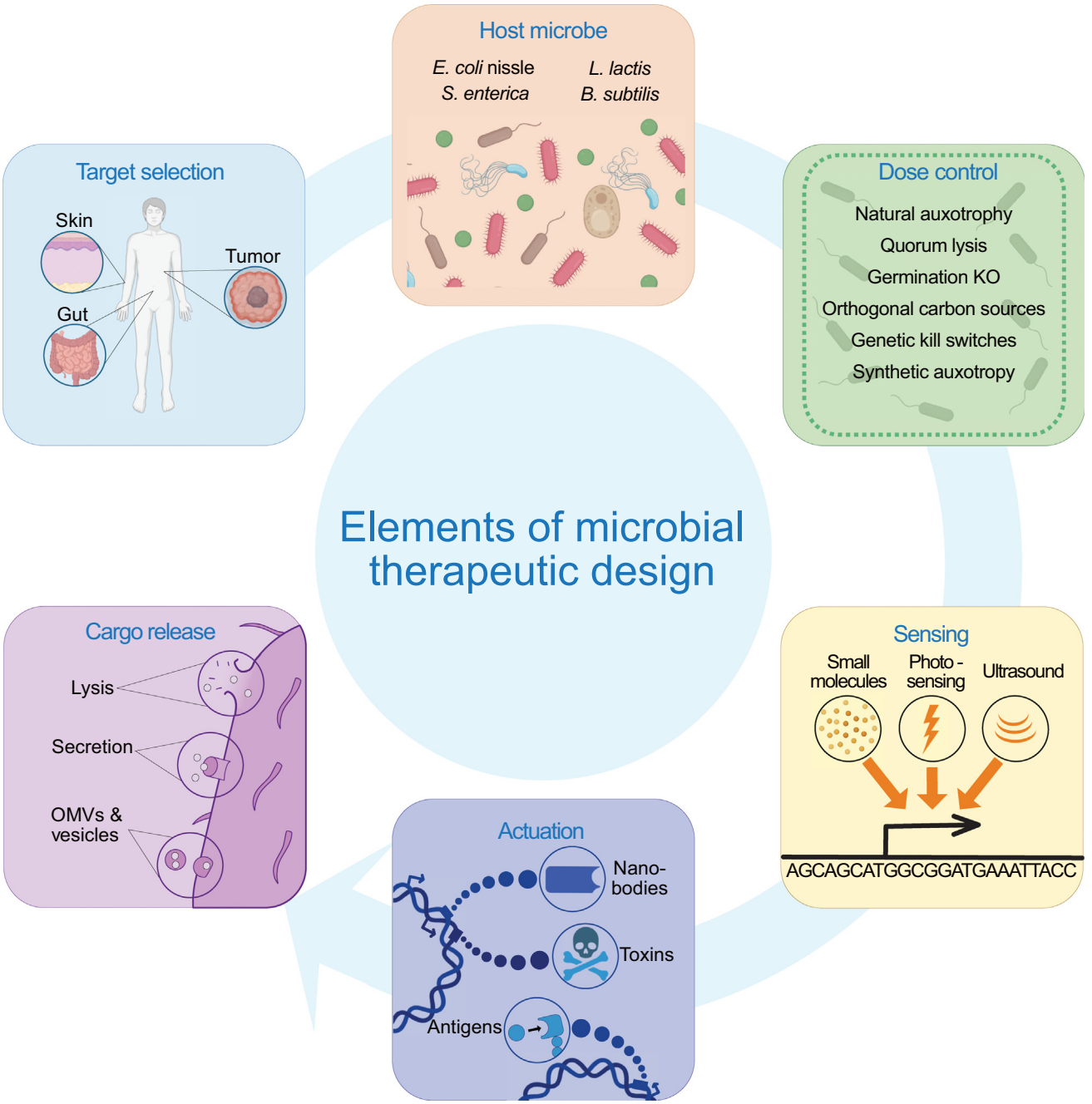

Figure 1. Elements of microbial therapeutic design. The development of engineered microbial therapeutics consists of numerous design decisions that cover target selection, microbial platform, biosynthesis of cargo, and the associated genetic modifications. Target selection: common target microenvironments are regions where microbes exhibit natural tropism such as the gut, skin, and tumor cores. Host microbe: while many microbes and microbial consortia have been formulated into therapeutics, the number of microbial candidates with existing and robust genetic engineering tools is limited. The microbes listed have seen genetically modified variants used in clinical trials. Dose control: to increase safety, a series of dosage control techniques can be utilized to either eliminate off-target microbes or cull high population densities to sensitize dosage. Sensing: for systems that may exhibit toxicity or are built to function in targeted systems, sensing and response can be utilized, often in the form of transcriptional control. Actuation: generated cargo are often small molecules, nucleic acids, or proteins that serve diagnostic or therapeutic purposes. Cargo release: while not all engineered microbes deploy cargo outside the cell, those that do require the genetic tools necessary to release into the extracellular space. Secretion serves as a hallmark method for cargo release, whereas engineered lysis, surface display, and encapsulation are alternative options. Abbreviations: KO, knockout; OMV, outer membrane vesicle.

The field of engineered therapeutic microbes has continued to mature since this study was published. The work of Din *et al.* to prototype the SLC exemplified that through clever design and appropriate steps in preclinical translation, genetic circuitry can expand the capabilities of bacteria to address drug delivery challenges. Regarding the SLC, its therapeutic utility is most clearly evidenced by subsequent work from the Danino laboratory, who utilized it to deliver and release nanobodies [5]. Furthermore, the Hasty laboratory fine-tuned SLC temporal and spatial control [6] and also utilized the SLC to improve the stability of a co-dependent 'rock-paper-scissors' coculture [7]. New cell lysis methods have also been developed, such as using ultrasound to disrupt engineered gas vesicles [8]. Meanwhile, a new generation of engineered microbes have made their way towards the clinic. For example, Synlogic's phenylketonuria-treating microbial strain SYNB1934 has reached Phase 3 trials (https://investor.synlogictx.com/news-releases/news-release-details/synlogic-announces-positive-top-line-phase-2-data), serving as a modern benchmark for engineered microbial therapeutics. Additionally, Novome Biotechnologies has had sizable investments from Roche. Their enteric hyperoxaluria treatment NOV-001, which consists of an engineered microbe and its orthogonal prebiotic carbon source, had a successful Phase 1 study (http://novomebio.com/wp-content/uploads/2021/11/Novome-Reports-Positive-Results-from-NOV-001-Phase-1.pdf) demonstrating gut colonization upon prebiotic dosing. GenCirq has been formed with plans to commercialize the SLC technology but has not yet begun clinical testing to our knowledge.

For each target disease, and especially for cancer treatment, advances in safety and efficacy through genetic engineering can unlock the full potential of live engineered microbes as a therapeutic modality. The choice of genetic circuit is influenced by several design considerations for engineered bacterial therapeutics, including control of dose, sensing, actuation, and cargo release (Figure 1). Fortunately, many circuits are generalizable across different microbial chassis and can be paired. Those who pioneered the SLC have demonstrated the value of creating complementary genetic circuits; for example, to address observed off-target colonization of bacterial strains in mice, Danino and colleagues later controlled tropism using biosensors that respond to select small molecules that are considered indicative of a tumor environment [9]. Often, there is not just one solution to an issue; other strategies to provide spatiotemporal control of engineered microbial survival include traditional auxotrophy [10], kill switches [11], and synthetic auxotrophy [12,13]. As additional complimentary genetic technologies are developed, therapeutics leveraging multiple circuits may be appealing for overcoming various design challenges. However, multiple layers of circuitry may compete for cellular resources and predictive testing of circuit pieces may be disparate for different layers, presenting new design challenges.

A final consideration is that while this study and recent commercial efforts to engineer live microbial therapeutics have focused on cancer or gastrointestinal diseases, similar technologies could help address other challenges in modern medicine. For example, while the use of live attenuated bacteria as vaccines was first explored by Pasteur in 1879 [14], there has been limited investigation towards using synthetic biology and engineered bacteria to develop modern vaccines against bacterial pathogens. Approaches such as synchronized cell lysis may have the potential to enhance vaccine efficacy through improved antigen release or adjuvant-like immune stimulation from lysed bacterial materials. This may provide a pathway for addressing antibiotic resistance, amongst other vaccine applications. Given the vast set of opportunities and the pace of synthetic biology innovation, it is reasonable to suggest that a surge of microbial therapeutics may soon be making their way towards the clinic.

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Declaration of interests

A.M.K. is a co-founder and equity holder of Nitro Biosciences, Inc., and serves on the scientific advisory board of Wild Microbes, Inc.

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References

1. Vargason, A.M. and Anselmo, A.C. (2018) Clinical translation of microbe-based therapies: current clinical landscape and preclinical outlook. *Bioeng. Transl. Med.* 3, 124–137
2. Kintzing, J.R. *et al.* (2016) Emerging strategies for developing next-generation protein therapeutics for cancer treatment. *Trends Pharmacol. Sci.* 37, 993–1008
3. Din, M.O. *et al.* (2016) Synchronized cycles of bacterial lysis for in vivo delivery. *Nature* 536, 81–85
4. Danino, T. *et al.* (2010) A synchronized quorum of genetic clocks. *Nature* 463, 326–330
5. Chowdhury, S. *et al.* (2019) Programmable bacteria induce durable tumor regression and systemic antitumor immunity. *Nat. Med.* 25, 1057–1063
6. Miano, A. *et al.* (2020) Inducible cell-to-cell signaling for tunable dynamics in microbial communities. *Nat. Commun.* 11, 1193
7. Liao, M.J. *et al.* (2019) Rock-paper-scissors: engineered population dynamics increase genetic stability. *Science* 365, 1045–1049
8. Bar-Zion, A. *et al.* (2021) Acoustically triggered mechanotherapy using genetically encoded gas vesicles. *Nat. Nanotechnol.* 16, 1403–1412
9. Chien, T. *et al.* (2021) Enhancing the tropism of bacteria via genetically programmed biosensors. *Nat. Biomed. Eng.* 6, 94–104
10. Isabella, V.M. *et al.* (2018) Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria. *Nat. Biotechnol.* 36, 857–867
11. Rottinghaus, A.G. *et al.* (2022) Genetically stable CRISPR-based kill switches for engineered microbes. *Nat. Commun.* 13, 1–17
12. Mandell, D.J. *et al.* (2015) Biocontainment of genetically modified organisms by synthetic protein design. *Nature* 518, 55–60
13. Kunjapur, A.M. *et al.* (2021) Synthetic auxotrophy remains stable after continuous evolution and in coculture with mammalian cells. *Sci. Adv.* 7, eabf5851
14. Barranco, C. (2020) The first live attenuated vaccines. *Nat. Res.* 2021, S7