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Review

Synthetic Bacteria for Therapeutics

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Synthetic biology builds programmed biological systems for a wide range of purposes such as improving human health, remedying the environment, and boosting the production of valuable chemical substances. In recent years, the rapid development of synthetic biology has enabled synthetic bacterium-based diagnoses and therapeutics superior to traditional methodologies by engaging bacterial sensing of and response to environmental signals inherent in these complex biological systems. Biosynthetic systems have opened a new avenue of disease diagnosis and treatment. In this review, we introduce designed synthetic bacterial systems acting as living therapeutics in the diagnosis and treatment of several diseases. We also discuss the safety and robustness of genetically modified synthetic bacteria inside the human body.

Keywords: Synthetic biology, synthetic bacterium-based therapies, living therapeutics, disease diagnosis, metabolic diseases, cancer

Introduction

Synthetic biology, in which artificial biological systems are designed and constructed from an engineering standpoint [1], is receiving increased attention of late. Scalable and robust synthetic biological systems have been widely applied to the construction of bio-factories for the production of biofuels, commodity chemicals, and pharmaceutical molecules [2–6]. With these recent advances, more sophisticated and rational strategies have emerged for the implementation of synthetic bacteria capable of performing programmed tasks in the field of medicine (Fig. 1) [7–9]. Particularly, the development of synthetic microorganisms as a therapeutic tool is on the rise due to bacterial abilities such as quorum sensing and responding to environmental cues, giving them an advantage over existing methods such as chemotherapy and radiation therapy [10]. Synthetic microorganisms are micro-machines that have been designed and constructed to perform specific functions in the human body as living therapeutics [11, 12]. One of the advantages of living therapeutics over conventional therapies is that the complex machinery of bacteria can be programmed to perform complex tasks. Micro-machines in nature have a broad range of different sensors and actuators, and these components can be utilized to reprogram existing bacteria to transform them into living therapeutic agents. Living therapeutics are promising in terms of being more efficacious, flexible, and cost-effective than conventional methods [13]. For example, a small number of engineered bacteria can be used to improve outcomes in disease treatment, while traditional therapies require larger amounts to be effective [14]. Such progress could reduce drug dosage and production costs and prevent undesirable systemic side effects. In recent years, there have been many reports on synthetic biology-based therapies using engineered bacteria for the treatment and diagnosis of cancers [15–17] and infectious diseases [18, 19] as well as for novel drug development [20].

In this report, we review recent developments and applications of synthetic microorganisms for diagnosis and treatment. In addition, we will explore current safety concerns involved with the use of synthetic bacteria for clinical purposes, including horizontal gene transfer, the unintended release of synthetic organisms into the environment, and undesirable production of synthetic materials [21].

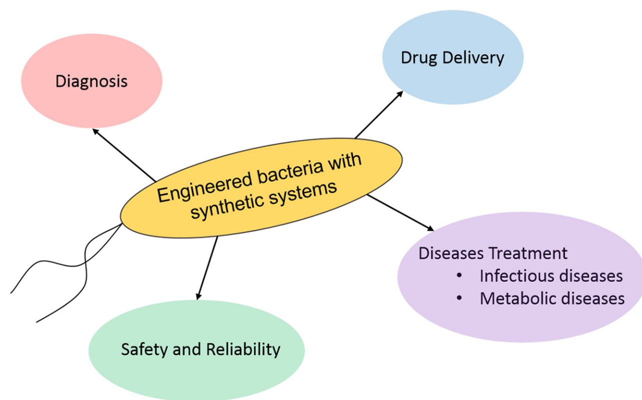

Fig. 1. Implementations of synthetic bacteria in the therapeutic field.

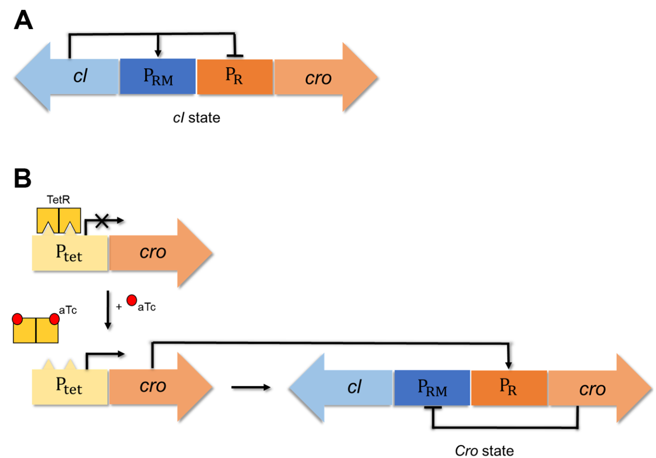

Fig. 2. Sensor and memory *cI/Cro* system.
(**A**) *cI* state. (**B**) In the presence of anhydrotetracycline (aTc), the TetR repressor conformational structure is altered, allowing the P_{tet} promoter to transcribe the *cro* gene; the cell is switched to a *cro* state.

Engineered Microorganisms for Therapeutic Applications

Diagnosis

The use of genetically programmed bacteria is a promising diagnostic strategy for many diseases [22]. Engineered bacteria are especially desirable in terms of efficiency and cost savings over traditional diagnostic methodologies [13, 23]. The advantages of living diagnostics include reducing outlays for detection devices and biochemistry procedures used in classical strategies and reducing the time required to produce results. Living therapeutics are also more flexible, as they can be programmed to recognize different diseases by changing the requisite biomarkers. Output signals occur in real-time and are highly correlated with the patient's disordered physiology. Here, we present several reports on engineered bacteria used for disease sensing or diagnosis.

Kotula *et al*. engineered *Escherichia coli* to employ a synthetic genetic sensor and memory to monitor exposure to antibiotics in the gut environment [24]. The memory system was derived from the competition between cI and Cro in the phage lambda that determines whether it engages in the lysogenic or lytic cycle. CI and Cro repress the expression of one another and can be used as a genetic switch. Under normal conditions, the *cI* gene represses expression of the *cro* gene, with the system in a lysogenic *cI* state (Fig. 2A). The sensor system consists of the *tetR* and *cro* genes. When the cell is exposed to tetracycline, or in some cases, anhydrotetracycline (aTc), the TetR repressor is released from the P_{tet} promoter that transcribes the *cro* gene, switching the cell to a lytic *cro* state in which the *cro* gene is continuously expressed, indicating exposure to antibiotics. In this study, engineered *E. coli* was administered to mice to sense and record aTc exposure in the gut. *E. coli* cells isolated from the fecal samples of aTc-administered mice were in the *cro* state, a positive signal for exposure (Fig. 2B). This system demonstrates one way in which synthetic bacteria can be used to detect and record environmental changes in vivo.

Daeffler *et al*. recently demonstrated a diagnostic tool for detecting inflammation in the colon (colitis) using gut-adapted probiotic bacteria [25]. The pathophysiological correlation between commensal bacterial sulfur metabolism and colitis in the gut is not well understood, but thiosulfate and tetrathionate metabolites could be produced by host inflammation [26, 27]. Daeffler and colleagues developed an engineered bacterium that could sense and report colonic thiosulfate and tetrathionate levels. Tetrathionate and thiosulfate sensors are based on two, two-component systems, TtrSR and ThsSR. TtrSR was derived from *Shewanella baltica* OS195, and ThsSR was obtained from the marine bacterium *Shewanella halifaxensis* HAW-EB4. The two-component system of TtrSR includes TtrS and TtrR, which express a membrane-bound sensor histidine kinase (SK) and a cytoplasmic response regulator (RR), respectively. Upon exposure to tetrathionate, TtrS phosphorylates TtrR, which, in turn, activates the promoter $P_{ttrB185\text{-}269}$ to trigger transcription of the *ttrBCA* tetrathionate reductase operon in the *S. baltica* genome [28]. Similarly, ThsSR is composed of ThsS and ThsR, and the presence of thiosulfate initiates the phosphorylation of ThsR by ThsS which activates the promoter $P_{phsA342}$ leading to the expression of the *phsABC*

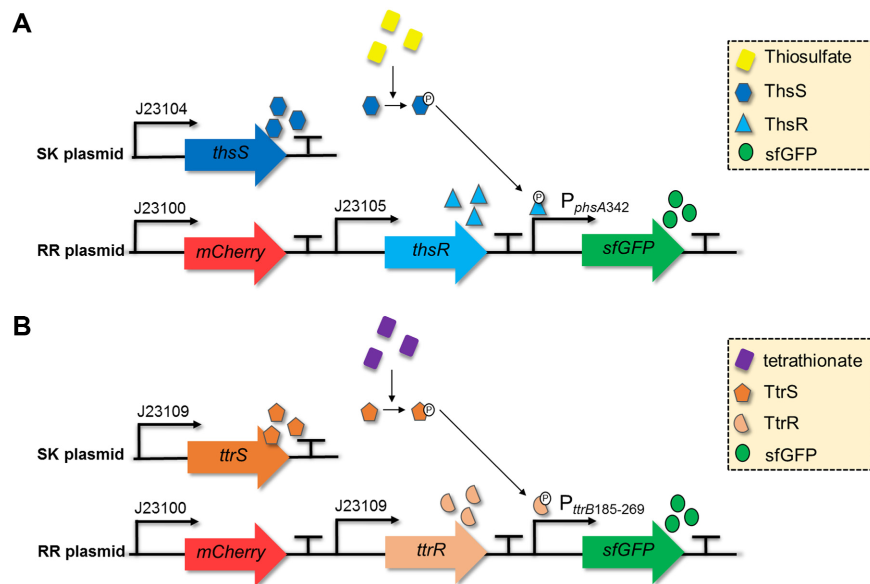

Fig. 3. Schematic designs illustrate the two-component system of (**A**) the ThsSR thiosulfate sensor, and (**B**) the TtrSR tetrathionate sensor.

gene encoding for thiosulfate reductase in *S. halifaxensis* [29]. These systems were artificially constructed, and the fluorescent reporter gene superfolder GFP (sfGFP) was placed downstream of the $P_{phsA342}$ and $P_{ttrB185\text{-}269}$ promoters induced by thiosulfate and tetrathionate, respectively (Fig. 3). They also inserted a mCherry gene downstream of a synthetic weak promoter J23100. The mCherry expression cassette was used as a reporter to distinguish the engineered sensor strains from native microbiota. The system was then transformed into the human probiotic strain *Escherichia coli* Nissle 1917. For in vivo evaluation of both sensors, Daeffler *et al*. fed dextran sodium sulfate (DSS) to experimental mice to induce colitis. DSS is a chemical colitogen with anticoagulant properties, and the DSS-induced colitis mouse is a well-established model for experimental colon inflammatory disease [30]. The engineered bacteria harboring the ThsSR system showed significantly higher sfGFP expression levels than normal mice. However, it was suspected that the TtrSR sensor might be inapplicable in an in vivo setting because the colitis mice showed relatively low activity from bacteria harboring the TtrSR sensor. Overall, this study suggests a potential sensor bacterium using thiosulfate as a novel biomarker for the diagnosis of colitis residing within complex colon and fecal samples.

There have also been reports on the use of engineered probiotics for cancer or tumor detection [31, 32]. Certain bacteria migrate and colonize selectively around tumors upon intravenous administration due to a preference for a micro-aerobic environment and metabolites such as amino acids produced by tumors [33, 34]. One study demonstrated an engineered probiotic *E. coli* Nissle 1917 designed to safely and selectively colonize at tumor sites and detect tumor metastasis [31]. The synthetic bacteria, named PROP-Z (programmable probiotics with lacZ), comprise several components. The first is a *luxCDABE* cassette integrated into the genome of *E. coli* Nissle 1917 for the generation of luminescent signals produced endogenously by bacterial luciferin and luciferase. The bacteria also contain plasmids that harbor an isopropyl-β-d-thiogalactopyranoside (IPTG)–inducible lacZ gene and dual-maintenance system (hok/sok and alp7) to ensure the prolonged stability of PROP-Z in a complex metastatic environment. The hok/sok maintenance system is a toxin-antitoxin system, where hok is a toxic substance, and sok is a short-lived antitoxin that regulates the *hok* gene at the translational level [35]. The hok/sok system will kill the cell upon plasmid loss. *hok* mRNA is more stable and persists longer in the cell than *sok* mRNA, leading to the production of the hok toxin that causes cell death [35]. Alp7 is an actin-like protein that pushes plasmids to the poles of the cell, guaranteeing equal DNA segregation during cell division [36]. The performance of PROP-Z was evaluated by testing on MC26-LucF (firefly luciferase) liver metastases-bearing mice after the intravenous injection of LuGal, a soluble conjugate of luciferin and galactose that can be converted to luciferin by lacZ (Fig. 4).

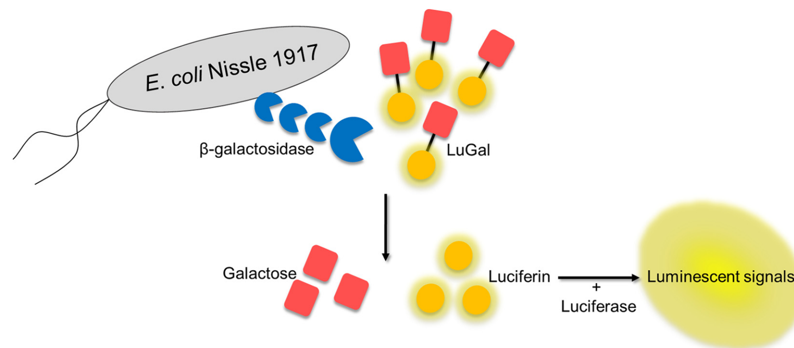

Fig. 4. PROP-Z (programmable probiotics with lacZ) colonizes tumors and detects liver metastasis in urine.
LuGal is broken down to galactose and luciferin by β-galactosidase produced by engineered PROP-Z. Luciferin is detected by a luciferase assay.

Subsequently, luciferin was detected by a luciferase assay as a signal for diagnosis of liver metastasis. This highly sensitive method requires only a small concentration of bacteria in diagnostic applications. With the improvements in synthetic tools, these living diagnostic approaches could be practically applied to enhance early detection of cancer and other diseases.

In general, the diagnostic studies mentioned above represent the ability of synthetic bacteria to sense, record, report environmental changes in vivo, and selectively detect tumors within complex samples. This leads to higher probabilities of detection in less time, and early diagnoses of disease increase the chances of successful treatment.

Drug Delivery

Synthetic microbes could provide a more efficient drug delivery system than current systemic treatments. Bacteria can be engineered to travel to a desired location and deliver one or more therapeutic agents produced by these bacteria for the treatment of one or more diseases simultaneously, with the promise of potent and efficient therapeutic effects. Bacteria migrate towards attractants, and this capacity is enabled by modules both for sensing chemicals and moving towards the attractants. Bacterial sensors (receptors) can be engineered to detect chemicals such as those produced at disease sites and monitor disease conditions or severity. As such, they can also be engineered to produce appropriate drugs metabolically [37]. Living therapeutics thus have the potential to act as targeted therapies necessitating reduced dosages, thereby limiting undesired systemic side effects. Genetically modified bacteria can be produced at a low cost since they can replicate exponentially on cheap carbon sources. In contrast, conventional drug manufacturing requires specialized filters and high-end, expensive analytical tools.

The use of synthetic microbes for drug delivery has been demonstrated in many reports [14, 19, 38]. *Lactococcus lactis* has been engineered to deliver anti-tumor necrosis factor (TNF)-α antibody for the treatment of chronic inflammatory bowel disease (IBD) [14]. IBD results from a collapse of tolerance to bacterial flora in the intestinal lumen [39], and *L. lactis* is a non-pathogenic bacterium that can specifically deliver the drug to the lumen of the gut [40]. To test its capacity in the treatment of a murine model for IBD, *L. lactis* was genetically engineered into a drug delivery vehicle that produced monovalent and bivalent murine (m)TNF-neutralizing antibodies. Since the monovalent and bivalent antibodies were anti-TNF, single-domain antibody fragments derived from heavy-chain camelid antibodies, a therapeutic agent used for murine rheumatoid arthritis, they were produced as a recombinant protein by the bacteria. This antibody-secreting *L. lactis* strain was administrated to chronic colitis-bearing mice through an intragastric catheter. The bivalent antibodies proved to be highly efficacious for the neutralization of both soluble and transmembrane TNFs even at low levels of administration. However, monovalent antibodies were found to be less effective in neutralizing transmembrane TNF than bivalent antibodies. This bacterial therapeutic strategy could mitigate the drawbacks inherent to systemic treatment of IBD patients (for whom Infliximal, a monoclonal, chimeric anti-TNF antibody is widely prescribed) such as adverse systemic side effects and high treatment cost.

Hamady *et al*. used the human commensal *Bacteroides ovatus* to produce human keratinocyte growth factor-2 (KGF-2) to treat IBD [38]. *Bacteroides* is an anaerobic genus found in the intestinal tract. *B. ovatus* specifically localizes to the mucin layer coating the colonic mucosa and uses the plant polysaccharide xylan as its sole energy source. KGF-2 is essential for intestinal epithelial cell proliferation,

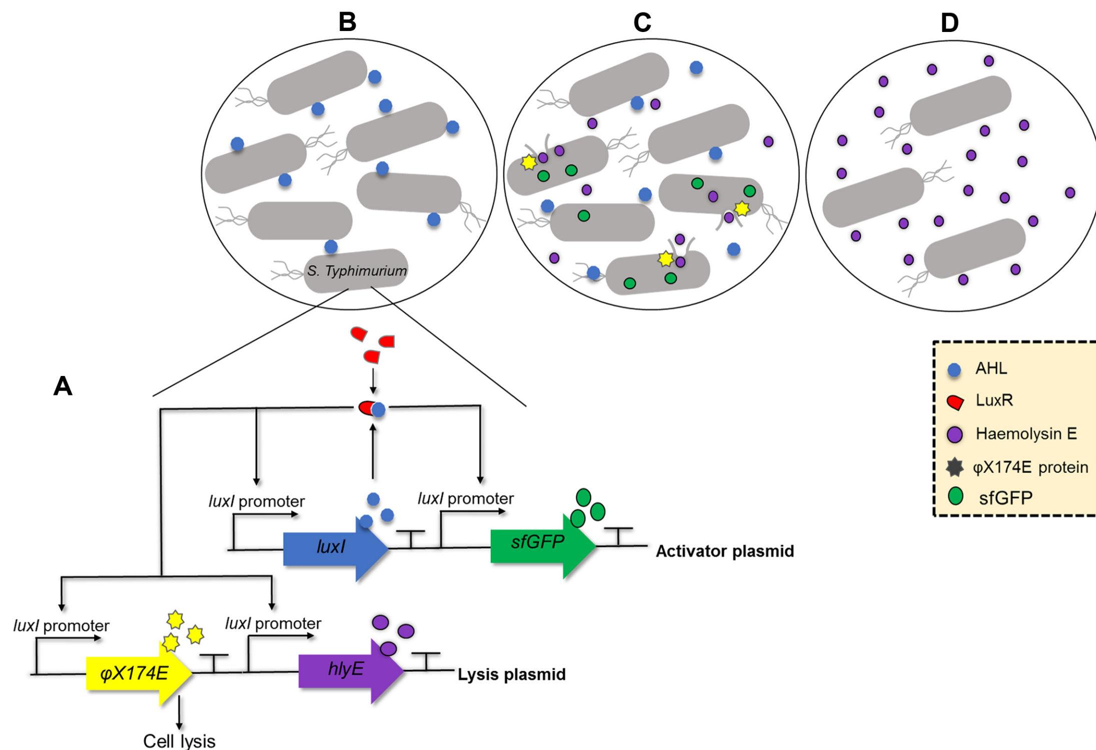

Fig. 5. Synchronized lysis circuit of *S. Typhimurium* releases the anti-cancer drug hemolysin E.
(**A**) AHL molecules that were produced by the *luxI* gene on an activator plasmid bind to LuxR synthesized by *S. Typhimurium*. LuxR-AHL complex binds to and transcriptionally activates the *luxI* promoter, which auto-synthesizes AHL and triggers the expression of *φX174E*, *hlyE*, and *sfGFP*. (**B**) AHL diffuses to nearby cells. (**C**) When AHL concentration reaches a quorum threshold in the bacterial population, cell lysis occurs for the release of hemolysin E. (**D**) A small number of surviving bacteria continue to grow and re-start the process in a cyclical manner.

homeostasis, and mucosa repair [41]. In the Hamady study, an enterotoxin secretion signal gene originated from *B. fragilis* and the human *kgf-2* gene were integrated into the genome of *B. ovatus* under the *B. ovatus* xylananse operon promoter. Engineered *B. ovatus* secretes active KGF-2 upon encountering xylan. This bacterium was evaluated in DSS-induced mice by oro-gastric gavage of 2×10^8 CFU of *B. ovatus* alongside 30 mg/ml xylan supplemented in drinking water. Treated mice showed a 30% to 40% increase in colonic epithelial cell proliferation and a significantly higher number of mucin-producing goblet cells compared with control mice. Treated mice also showed increased inhibition of colitis development such as less reduction in colon length, a 45% reduction in epithelial damage and inflammatory infiltrate scores, and lower levels of pro-inflammatory cytokines such as TNFα, interleukin (IL)-1β, and IL-6. *B. ovatus* is considered a safe drug delivery vehicle as it is anaerobic, meaning the engineered strain cannot live outside of the host body. This provides a biosafety means of preventing horizontal gene transfer. The Hamady study strongly supports the potential of bacteria-based therapies.

Remarkably, a recent study by Din *et al*. demonstrated that an attenuated *Salmonella enterica serovar Typhimurium* strain could be used for drug delivery using a synchronized lysis circuit (SLC) [19]. *S. Typhimurium* was programmed to lyse and release anti-cancer drugs in a cyclical manner; specifically, the engineered strain harbors two plasmids: an activator plasmid containing the *luxI* gene that synthesizes the acyl-homoserine lactone (AHL) autoinducer and the fluorescent superfolder protein sfGFP; and a lysis plasmid with a lysis gene from the φX174E bacteriophage and the pore-forming, anti-tumor toxin gene *hlyE* obtained from *E. coli* MG1655 (hemolysin E) (Fig. 5A). All genes are placed under the control of the common *luxI* promoter, which is activated by AHL-bound LuxR. A LuxR homologue is genetically encoded by *S. Typhimurium*, and the AHL-LuxR complex binds to the promoter and transcriptionally activates *luxI* expression to auto-synthesize AHL via a positive feedback loop. Due to the increase in LuxI enzymes producing AHL, the concentration of AHL gradually increases and diffuses to nearby cells. When

AHL concentration reaches a quorum threshold in the bacterial population, the cell lysis circuit is activated and releases the hemolysin E porin in synchronization mode (Fig. 5). Most of the bacterial population died, but a small number of surviving bacteria continued to grow and were able to re-start the process (synchronized drug lysis) in a cyclical manner. For the purposes of improved cancer treatment, two additional strains were developed for immune recruitment and induction of tumor cell apoptosis. When these three strains were injected together into mice bearing subcutaneous tumors, the treated mice showed a slower growth rate of tumors. Interestingly, the combinatorial injection of the engineered SLC strains and oral administration of 5-fluorouracil (5-FU) for chemotherapy reduced tumor activity by 30% and increased survival times of mice with colorectal metastases by 50%. The synchronized drug delivery system proved to be a prospective drug delivery strategy since it did not require re-intake of drugs, and it did reduce the probability of systemic inflammation due to oscillatory population control.

Other applications of synthetic live bacteria for drug delivery purposes have also been reported [21, 37]. A crucial advantage of synthetic bacteria drug delivery systems is their ability to minimize or avoid the risk of systemic side effects inherent to traditional systemic drug administration. However, in order to safely use living therapeutics, more research is necessary to understand the complex interactions of microbial systems with their host.

Engineered Microbes against Diseases

Given the benefits of microbial therapeutics for drug delivery and disease diagnosis, it is not surprising that interest in engineered bacteria has extended into infectious and metabolic disease research [42−44]. *E. coli* Nissle 1917 was modified to express cholera autoinducer 1 (CAI-1) to inhibit infection by *Vibrio cholerae* [45]. In this bacterial quorum sensing system, at high cell density, proteins CAI-1 and AI-2 (autoinducer 2 synthesized by the *luxS* gene that already presents in *E. coli* Nissle) are produced externally and bind to their sensor proteins LuxP/LuxQ and CqsS, respectively. These bindings alter internal kinases and suppress the transcription of four short, non-coding small RNAs (sRNAs) (Qrr1-4 or quorum regulatory RNAs), preventing the translation of HapR mRNA [46]. Without sRNAs, the HapR regulator is transcribed, which down-regulates the expression of virulence genes, indicating that simultaneous expression of CAI-1 and AI-2 could prevent *V. cholerae* virulence. As described in the study, the CAI-1-expressing Nissle was constructed, and two-to-three-day-old mice were infected with *V. Cholerae* 8 h after being fed the engineered Nissle bacteria (10^9 cells). Pretreated mice exhibited a higher survival rate (92%) than non-pretreated mice.

Saeidi *et al*. and Hwang *et al*. reported on engineered *E. coli* Nissle 1917 for the treatment of infectious diseases, specifically *Pseudomonas aeruginosa* gut infection [47, 48]. Saeidi and colleagues engineered Nissle with a synthetic quorum-sensing system that inhibited 99% of *P. aeruginosa* growth in mixed culture with Nissle and reduced the formation of *P. aeruginosa* biofilm by 90% [48]. Modified *E. coli* Nissle harbors a *lasR* gene expressing the transcriptional factor LasR that is controlled by the *tetR* promoter. LasR binds to AHL molecules produced by *P. aeruginosa*, leading to the transcription of the *pyoS5* gene that encodes for pyocin S5, a strong bacteriocin against *P. aeruginosa*, and the *E7* lysis gene for disrupting the *E. coli* membrane to release pyocin S5 once a threshold cell density of *P. aeruginosa* is reached. Since AHL is only produced by *P. aeruginosa*, cell lysis and pyocin release occur only if *P. aeruginosa* is present.

Saeidi's team did not evaluate their synthetic therapeutic bacterium in an animal model, but in 2017, Hwang *et al*. introduced an improved version of previously engineered *E. coli* Nissle (Fig. 6) to test in mice [47]. The *E. coli* Nissle strain used in the study had its *dadX* and *alr* genes deleted. These two genes encode for alanine racemases essential for D-alanine metabolism. This *E. coli* Nissle Δ*dadX*Δ*alr* strain thus requires supplemental D-alanine for growth and contains a plasmid that harbors the *alr* gene to complement the deletion and facilitate *E. coli* survival. This strategy could mitigate the risk of horizontal gene transfer of antibiotic resistance genes, resulting in better biocontainment. In addition to the genetic circuits of the engineered Nissle designed by Saeidi *et al*., Δ*dadX* Δ*alr* Nissle was programmed to express dispersin B (DspB), which degrades biofilms created by *Actinobacillus actinomycetemcomitans*. DspB hydrolyzes 1,6-N-acetyl-D-glucosamine, an adhesin polysaccharide that stabilizes biofilm formation and enhances its integrity, to facilitate biofilm destabilization. The release of pyocin S5 and DspB after cell lysis by lysin E7 through quorum sensing of AHL resulted in 98% inhibition of *P. aeruginosa* infection in mice. This suggests that this synthetic bacterium is highly sensitive, efficacious, and safe for protection against *P. aeruginosa* infection.

Engineered bacteria have also been used to inhibit a class of sexually transmitted diseases caused by *Chlamydia trachomatis* [18]. *C. trachomatis* is a widespread human

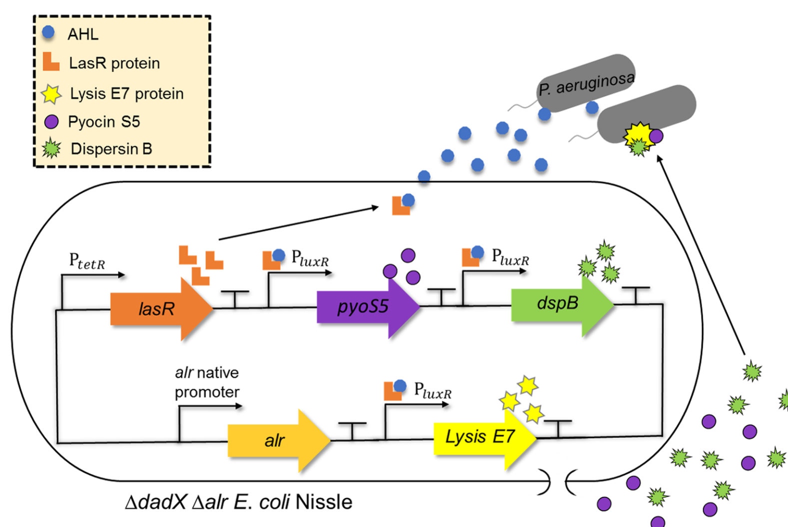

Fig. 6. Engineered Δ*dadX*Δ*alr E. coli* Nissle inhibits *P. aeruginosa* infection.
The transcriptional factor LasR expresses and binds to AHL molecules produced by *P. aeruginosa*. LasR-AHL binding activates P_{luxR}, triggering the expression of *pyoS5* and the *E7* lysis gene. When the cell density of *P. aeruginosa* reaches a certain threshold, the *E. coli* membrane is disrupted, consequently releasing pyocin S5 and dispersin B.

pathogen, for which vaccine and microbicide development has been underway for 100 years. As of yet, no vaccines have been developed that can fully inhibit *C. trachomatis* infection. The M13 bacteriophage was designed to carry two functional peptides, an integrin-binding peptide (RGD) that facilitates integrin-mediated endocytosis to internalize into eukaryotic cells, and a segment of the polymorphic membrane protein D (PmpD) that interrupts *C. trachomatis* infection and propagation. RGD motifs were expressed on the M13 pVIII major coat proteins, while five copies of the PmpD peptide were presented by pIII minor coat proteins. Although the mechanism of PmpD action is not fully understood, the administration of M13-RGD-PmpD in vitro resulted in a significant reduction of *C. trachomatis* infection in both HeLa and primary endocervical cells.

Administration of engineered bacteria could also treat metabolic diseases, including type 1 diabetes (T1D), which is caused by an auto-immune response to β cell-specific antigens that leads to the destruction of insulin-producing β-cells [49]. A recent report demonstrated the use of engineered *L. lactis* to reverse T1D in non-obese diabetic (NOD) mice [50]. As they wanted to target only β-cell-reactive T cells and not affect antigens unrelated to diabetes, the bacteria were designed to produce autoantigen-specific antigens. Glutamic acid decarboxylase (GAD65) is the main autoantigen in T1D [51], and anti-GAD65 autoantibodies are found in most patients. To prevent the degradation of autoantigens in the acidic gastric environment, researchers employed *L. lactis*, which can migrate to the human gut without triggering intestinal immune responses [40]. *L. lactis* was constructed to harbor a plasmid expressing human $GAD65_{370-575}$ autoantigen and the anti-inflammatory cytokine IL-10 to restore immune tolerance to β cell-specific antibodies in T1D patients. The efficacy of *L. lactis* was evaluated by orally administering it to recent-onset NOD mice five days a week for six weeks. In addition to *L. lactis*, systemic, low-dose anti-CD3 was also given for the first four days to provide time for the antigen-specific immune response to reach its peak around 10 to 14 days into the treatment period; anti-CD3 results in an instantaneous freezing of immune responses. *L. lactis* expressing $GAD65_{370-575}$ and IL-10 in combination with anti-CD3 preserved β-cells and prevented T1D progression in NOD mice. Specifically, treated mice showed a 67% reversal of diabetes that was maintained for eight weeks after the termination of treatment.

Some live therapies for obesity have been evaluated by incorporating engineered bacteria into the gut microbiota to fine-tune the production of N-acyl-phosphatidylethanolamines (NAPEs), the immediate precursors of the N-acylethanolamides (NAEs) family of lipids [52, 53]. NAEs are known satiety factors, and biosynthesis of NAPEs in the intestinal tract can reduce food intake [54]. It has been observed that NAPE production is reduced in obese individuals. Chen *et al.* developed a NAPE-expressing *E. coli* Nissle 1917 bacteria to compensate for this reduction

[53]. The engineered Nissle harbored a plasmid expressing the *At1g78690* gene from *Arabidopsis thaliana* that codes for an N-acyltransferase that catalyzes the synthesis of NAPEs. Administration of engineered bacteria at 5×10^9 CFU/ml of drinking water for eight weeks in mice fed a high-fat diet resulted in less weight gain and fat mass accumulation than in control mice. This moderating effect of modified Nissle was maintained for up to six weeks after the end of administration, suggesting that live synthetic bacteria-based therapy holds promise in the treatment of obesity.

Recent advances in synthetic biology have been applied to cancer therapy, as well, by manipulating bacteria to target tumors and respond to tumor microenvironments [15, 55]. Park *et al*. described a tumor-targeting strategy using cell-surface display in attenuated *Salmonella typhimurium*, which is capable of inhibiting tumor growth [56]. A plasmid carried by the attenuated *S. typhimurium* deficient in ppGpp synthesis contains an arginine-glycine-aspartate (RGD) sequence inserted into the third transmembrane domain of the *ompA* gene that encodes for outer membrane protein A (OmpA). The RGD peptide exhibited by *S. typhimurium* can specifically bind to αvβ3 integrin, which is generally overexpressed in tumor cells and tumor endothelial cells, to suppress tumor growth. Although the mechanism of suppression by *S. typhimurium* is not fully known, it is assumed that the lipopolysaccharide (LPS) released within the tumor cells during *S. typhimurium* infection induces immune system first responder macrophages and dendritic cells via the production of tumor necrosis factor α (TNF-α) and IL-1β. In vivo tests of this system in a mice model resulted in the regression of αvβ3-overexpressing xenograft tumors. As the engineering of bacterial behavior continues to develop, more complex and sophisticated forms of bacterial cancer treatments are on the horizon [57−59].

The studies described here all demonstrated high success rates in disease prevention and treatment with small numbers of engineered bacteria. Some of these bacteria can specifically colonize and secrete therapeutic agents directly at disease (tumors/cancers) sites without affecting other body systems. Synthetic living therapeutics are also more effective over the long term than traditional treatment

Table 1. Synthetic bacteria-based methods for bio-therapy.

Applications		Diseases	Bacteria Strain	Description	References
Diagnosis		Colon inflammation	*E. coli* Nissle 1917	Thiosulfate and tetrathionate sensors	[25]
		Cancer	*E. coli* Nissle 1917	Liver metastasis detection in urine	[31]
Drug delivery		Tumor	*Salmonella enterica serovar Typhimurium*	Secretion of hemolysin E, a pore-forming anti-tumor toxin, in synchronized cycles	[19]
		Chronic inflammatory bowel disease	*Lactococcus lactis*	Secretion of monovalent and bivalent murine (m)TNF-neutralizing antibodies	[14]
		Colon inflammation	*Bacteroides ovatus*	Secretion of human keratinocyte growth factor-2	[38]
Treatment	Infectious diseases	*Vibrio cholerae* infection	*E. coli* Nissle 1917	Inhibit infection by producing cholera autoinducer 1 (CAI-1) (assisted by AI-2) showing a survival rate of 92%	[45]
		Pseudomonas aeruginosa gut infection	*E. coli* Nissle 1917	Inhibit infection by producing pyocin S5 (assisted by AHLs and E7 lysis protein) showing a survival rate of 99%	[47, 48]
		Chlamydia trachomatis infection	M13 bacteriophage	Inhibit infection by producing integrin binding peptide (RGD) and a segment of the polymorphic membrane protein D (PmpD)	[18]
	Metabolic diseases	Type 1 diabetes	*Lactococcus lactis*	Preservation of β-cells through production of T1D autoantigen GAD65$_{370-}$575 and anti-inflammatory cytokine IL-10	[50]
		Obesity	*E. coli* Nissle 1917	Embedding of N-acylphosphatidylethanolamines (NAPEs)-synthesizing enzyme to reduce food uptake for treatment of obesity.	[53]
		Cancer	Attenuated *Salmonella typhimurium*	Colonizes tumor by expressing an RGD motif fused within the transmembrane protein OmpA, subsequently suppressing tumor growth by the release of TNF-α and IL-1β	[56]

strategies because these bacteria are capable of residing in the host and continuously treating disease in response to the disease's chemical signals. Current applications of synthetic bacteria for disease diagnosis and treatment are provided in Table 1.

Safety of Engineered Therapeutic Bacteria and Reliability of the Synthetic Systems Employed Therein

With regards to safety, *Lactobacillus*, *Lactococcus, Bacteroides* genera, and *E. coli* Nissle are all good chassis for implementing synthetic systems. These commensal bacteria are nontoxic and considered harmless for in vivo applications owing to the fact that they are already used as probiotics [60−62]. It would thus be easier to gain acceptance from government agencies and generate public consensus supporting the use of these genetically engineered bacteria in medicine than it would be for novel or uncommon strains [63]. Because of their reliability as probiotics, synthetic biologists have focused on these bacteria and programmed them to serve as living diagnostics, such as reporting environmental signals in the mammalian gut or enabling tumor detection and imaging [14]. However, in some cases, commensal bacteria are no more effective than other bacteria for targeting specific sites inside the human body. Certain organisms have the propensity to colonize specific body niches, with attenuated strains of *S. typhimurium* as an example; these bacteria have a natural preference for colonizing tumor cells with a higher affinity than *E. coli* [56]. Additionally, it is essential to understand safety and containment issues arising from the use of modified bacteria [64, 65]. Plasmid-free bacteria are preferred to prevent horizontal gene transfer because any bacteria harboring a plasmid-expressed recombinant protein could behave differently or mutate in a dynamic and unstable environment like the human body [21]. Furthermore, in vivo applications do not allow the same level of control over strain efficiency as in vitro settings, and could lead to undesirable systemic effects. Accordingly, several approaches have been recommended, such as the self-destruction of synthetic bacteria or supplementation requirements. When the treatment is completed and the presence of the therapeutic strain is no longer required, such strains are programmed to initiate cell lysis leading to death. To ameliorate the risk of intentional or unintentional leakage of synthetic organisms outside of the laboratory or host, it is recommended that strains be re-constructed to render them incapable of surviving outside the host, or in need of a specific supplement to propagate. Biosafety concerns regarding the use of genetically modified organisms in general and specifically in clinical settings must be addressed, and the likelihood of environmental containment and horizontal gene transfer must be reduced to virtually zero. If these conditions can be met, living therapeutics have a promising future.

Microbial cells genetically programmed using synthetic biological techniques have been shown to be effective therapeutic agents in diagnosing, targeting, and treating a range of diseases. So far, several proof-of-concept systems have been designed and validated, but next-generation therapy will be more complex due to the need to integrate modules for diagnosis, signal integration, and drug delivery. As our understanding of the connection between the human microbiome and health or disease increases, opportunities for developing new biomarkers and therapeutics are expected to increase as well. We are closer than ever to realizing clinical applications of synthetic bacteria through the integration of traditional and microbial ecological approaches. However, when using such living therapies in the human body, special attention must be paid to safety and biocontainment. Further research on safety needs to be done before moving into clinical applications of this innovative therapy.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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