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Review article

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Bacterial therapies at the interface of synthetic biology and nanomedicine

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Abstract

Bacteria are emerging as living drugs to treat a broad range of disease indications. However, the inherent advantages of these replicating and immunostimulatory therapies also carry the potential for toxicity. Advances in synthetic biology and the integration of nanomedicine can address this challenge through the engineering of controllable systems that regulate spatial and temporal activation for improved safety and efficacy. Here, we review recent progress in nanobiotechnology-driven engineering of bacteria-based therapies, highlighting limitations and opportunities that will facilitate clinical translation.

Sections

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Key points

- Synthetic biology has brought about the rapid development of live bacteria-based therapeutics in the last two decades.
- However, using live bacteria presents challenges for the translation of proof-of-concept work into the clinic.
- The integration of synthetic biology and nanomedicine could overcome some of the challenges faced by bacterial therapy.

Introduction

Living cells can be engineered as new medicines that dynamically respond to external and environmental cues with therapeutic intervention. Synthetic biology approaches enable the design of sense-and-respond genetic circuits, whereas techniques from nanotechnology allow for the creation of stimuli-responsive systems through chemical and molecular engineering[1–3]. Thus far, an array of cell types has been developed for use as drugs, including stem cells, chimeric antigen receptor (CAR) T cells, yeast, algae and bacteria[4–6]. Owing to the growing appreciation of the role that microorganisms have in human health[7–9] and recent developments in synthetic biology tools[10,11], bacteria have garnered considerable interest as new therapeutic agents to treat various diseases, including inflammation, infectious diseases, metabolic disorders and cancer[12–23] (Box 1).

Louis Pasteur was the first to produce a laboratory-developed vaccine for chicken cholera by using attenuated *Pasteurella multocida* — a live bacterial product — in 1879 (ref. 24). Vaccines for cholera in humans were also the first recorded use of live strains of *Vibrio cholerae* as early as 1884 (ref. 25). Decades later, Bacillus Calmette–Guérin (BCG), a live attenuated strain of *Mycobacterium bovis*, was used as a tuberculosis vaccine developed by Albert Calmette and Camille Guérin in 1921 (refs. 26,27). More recently in 1989, the US Food and Drug Administration (FDA) approved the attenuated strain *Salmonella typhi* Ty21aA as a typhoid vaccine[28,29], and BCG was approved in 1990 to treat high-risk non-muscle invasive bladder cancer, which was the first non-vaccine use of a live bacterial therapy approved by the FDA for cancer[30,31]. Coming full circle to Pasteur's work, a live attenuated strain of *Vibrio cholerae*, marketed as Vaxchora, was approved by the FDA in 2016 as a cholera vaccine[32,33].

In 2022, Rebyota, a broad-consortium microbiota suspension to prevent the recurrence of *Clostridioides difficile* infection, became the first faecal microbiota product and another non-vaccine use of live bacterial therapy approved by the FDA, signalling a new era of microorganism-based therapies that go beyond probiotic dietary supplements[34–36]. In 2023, the FDA approved the first faecal microbiota pill for oral administration, Vowst, also for the prevention of recurrent *Clostridioides difficile* infection, indicating the steady increase in the regulatory approval and use of live microorganisms in the clinic[37–39]. However, numerous trials to date have yet to achieve robust clinical efficacy[40]. One of the major hurdles for bacterial therapy is the dual nature of bacteria as replicating and immunostimulatory medicines. Although rapid replication of bacteria amplifies the therapeutic effects from in situ production of drugs, it can also lead to off-target accumulation and toxicity. Additionally, the immunogenicity of bacteria can either act as an adjuvant for immunotherapy or cause cytokine storm and sepsis[41,42]. These aspects are challenging to the host immune system; it must overlook bacteria in the target diseased tissue to maintain the therapeutic effects but clear them from healthy environments to maintain homeostasis.

Delivery strategies are crucial to addressing the challenges en route and in situ to facilitate the translation of bacterial therapies from bench to bedside (Fig. 1). A major focus thus far has been the attenuation of bacteria to minimize systemic effects, but this approach yielded limited success in several clinical trials (Box 2). In addition to synthetic biology strategies, bacteria-based systems have adopted advances in nanotechnology and molecular engineering for the development of nanoparticle platforms for drug delivery[2,3]. In this Review, we use nanomedicine as a guidepost to highlight the similarities as well as the unique challenges and opportunities that arise from transforming bacteria into therapeutics. We then examine advances at the interface of synthetic biology and nanotechnology through the engineering of bacteria that could overcome these challenges. We conclude by offering an outlook on bacterial therapies and potential paths forward through further integration of synthetic biology and nanotechnology approaches for bacterial engineering.

Bacteria and nanoparticles

Paul Ehrlich's vision of a 'magic bullet'[43] has driven the investigation of drug-delivery systems that can specifically target diseased sites. Advances in nanomedicine over the last few decades have brought this vision closer to reality by controlling the pharmacokinetics and pharmacodynamics (PK/PD) of therapeutic agents through nanoparticle-based systems that can prolong circulation lifetimes, alter biodistribution and control the release kinetics of drugs. However, the dynamic nature of in vivo environments still poses a challenge to even the most advanced drug-delivery systems. Engineered bacteria can overcome some of these challenges through replication and complex sense-and-respond genetic circuits while adapting innovative approaches from the field of nanomedicine. Moving forward, it is essential to understand the properties that contribute to the pharmacological performance of bacteria. Drawing a comparison with nanomedicine, we discuss bacterial properties that affect their function as therapeutics (Fig. 2).

Scaffold

There are approximately 30,000 formally named species of bacteria that have a range of shapes and sizes[44]. Although nanoparticles are generally categorized by their chemical nature (for example, lipid-based, polymeric, inorganic), bacterial scaffolds can be broadly divided into two types based on their cell wall structure: Gram-positive and Gram-negative. Gram-positive bacteria possess a lipid membrane covered with a thick cell wall (20–80 nm) composed of peptidoglycan and teichoic acid. Gram-negative bacteria have an inner lipid membrane with a relatively thin layer (5–10 nm) of peptidoglycan surrounded by an outer lipid membrane with lipopolysaccharides and lipoproteins.

The composition of the outermost layer is an important factor in determining bacteria–host interactions. Bacterial immunogens known as pathogen-associated molecular patterns (PAMPs) are recognized by an array of Toll-like receptors (TLRs) that serve as the first line of defence against invading pathogens[45]. In humans, ten different TLRs arm innate immune cells with the ability to detect and respond to a variety of PAMPs depending on the subcellular location of the receptor — generally, intracellular TLRs recognize viral and bacterial nucleic acids, whereas surface-bound TLRs mainly identify membrane components of extracellular bacteria. At the outermost surface, Gram-positive bacteria present teichoic acid and peptidoglycans that are potent

agonists of TLR2 (refs. 46,47), whereas exposed lipopolysaccharides on Gram-negative bacteria typically stimulate activation of TLR4 (ref. 48). Moreover, TLR5 recognizes the flagella that some species of both Gram-positive and Gram-negative bacteria possess for motility[49]. In addition to the interaction between PAMPs and TLRs, the outermost layer of bacteria determines their susceptibility to the complement system, which serves as a first line of defence within innate immunity. The thick layer of peptidoglycan in the cell wall protects Gram-positive bacteria from the complement-mediated formation of the membrane attack complex, which may make them less suitable for immunocompromised patients. Therefore, consideration of the shared and differing PAMPs as well as other molecular compositions between strains during scaffold selection will have important implications on host immune responses.

Another consequence of membrane structures is the impact on therapeutic payload release (Box 1). Notably, Gram-negative bacteria face a greater challenge for extracellular protein secretion as these proteins must cross two lipid membranes[50]. In this case, expressed proteins are secreted through two separate steps that sequentially transport proteins to the periplasm and then across the outer membrane or through channels that span both membranes[51]. By contrast, Gram-positive bacteria can more readily secrete expressed proteins. However, both types of bacteria have evolved strategies to secrete cytoplasmic molecules to the immediate surroundings or inject them into the target cell, and these secretion systems can be repurposed to deliver therapeutic molecules[52–54]. Furthermore, strategies have been developed to improve the secretion efficiency of *Escherichia coli*, a popular model organism and Gram-negative bacteria[55]. For example, knocking out genes involved in the formation of cell envelope components results in leaky strains with improved secretion of exogenous proteins[56]. Overall, the choice of bacterial scaffold between Gram-positive and Gram-negative affects the release of therapeutics and their interaction with the host.

Lastly, an important criterion for selecting a suitable bacterial scaffold is the availability of genetic toolkits. Although *E. coli* has the largest array of tools available, there is a growing list of genetically tractable bacterial scaffolds, including Gram-positive bacteria (such as *Bacillus subtilis*, *Listeria* spp., *Clostridium* spp. and *Lactobacillus* spp.) and other Gram-negative bacteria (such as *Vibrio natriegens*, *Salmonella* spp., *Bacteroides* spp.)[57–59]. The bacterial genetic toolkits now include genome engineering techniques such as CRISPR-based gene editing, which can vastly improve efficiency and scale[60–62] and could be expanded to other bacterial scaffolds. Moreover, metagenomic engineering techniques, such as in situ conjugation, enable a community-wide modification of diverse taxa in their native environment[63]. Given the currently small number of bacterial strains being explored for therapeutic use, expanding the panel of bacterial scaffolds by developing genetic toolkits will help advance the use of bacteria as therapeutics.

Box 1

Mechanism of action and payload delivery with bacteria

Bacterial therapy can result in therapeutic effects via innate microbial function, stimulation of immune response and production of recombinant therapeutic molecules. For example, probiotics restore microbiome homeostasis, decrease gut inflammation and eliminate pathogens through their symbiotic relationship with the host[172]. Moreover, one of the earliest reports of cancer immunotherapy was the injection of bacteria, also known as Coley toxins, to induce potent immune responses to treat inoperable sarcoma[173,174]. Beyond the use of naturally occurring bacteria, recent advances in molecular biology have enabled genetic engineering to produce therapeutic molecules from bacterial scaffolds to enhance the desired response from the host. Therapeutic enzymes can be expressed in the cytoplasm of bacteria to convert prodrugs into cytotoxic products[175], degrade malignant metabolites[176] or metabolize biological waste into therapeutically relevant molecules in the targeted region[177]. This means of therapeutic delivery has matured enough to be used in clinical trials (NCT01562626 and NCT05764239)[178,179].

The effect of therapeutic payloads often requires their release into the extracellular environment or cytoplasm of target cells. One strategy to release therapeutic proteins is to fuse known bacterial secretion tags directly onto the payload[50]. The type III secretion system is one example that has been used to deliver effector proteins in the tumour milieu or directly into the cytoplasm of the target eukaryotic cell[180,181]. Alternatively, a quorum-sensing system has been used for protein release where bacteria are programmed to express a phage lysis gene for synchronized lysis once they reach a certain threshold density in a cyclic manner[182]. This synchronized lysis circuit was used to deliver a wide range of proteins, including toxins, peptides, chemokines, checkpoint inhibitors and nanobodies[182–185]. To deliver DNA and RNA molecules into the nucleus or cytoplasm, three major steps need to be considered: cell invasion, endosomal escape and therapeutic molecule release. This strategy can be achieved by either introducing virulence factors into extracellular bacteria[186] or using intracellular bacteria[187] that are programmed to lyse and release their therapeutic cargo from within the host cell[188,189].

Physical properties

The morphology of therapeutic agents can affect their interaction with the host environment. Nanoparticles and bacteria cover adjacent size regimes: nanoparticles are generally defined as particles ranging from 1 to 100 nm in diameter, whereas bacterial dimensions typically range from 100 nm to 10 µm. Most nanoparticles are spherical, but special fabrication techniques can produce nanoparticles with different shapes such as rods, wires, discs, cubes and stars. Similarly, different shapes of bacteria exist, including spheres, rods, spirals, commas and corkscrews. Both shape and size affect the interaction with host tissues, microenvironments, and cells and lead to varying means and rates of clearance[64]. For example, bacteria are more susceptible to clearance by phagocytosis and splenic filtration compared to nanoparticles owing to their larger size. However, the large size also excludes bacteria from renal filtration, which affects small nanoparticles (<15 nm)[65]. Cellular-level interactions also require remodelling of the plasma membrane of host cells to fit the geometric dimension and receptor organization required for downstream actions, such as endocytosis, phagocytosis and signal transduction, to activate a specific immune response. Though still in its infancy, manipulation of bacteria morphology has become of interest for bio-production applications[66,67], and these advances may benefit the development of bacterial therapies.

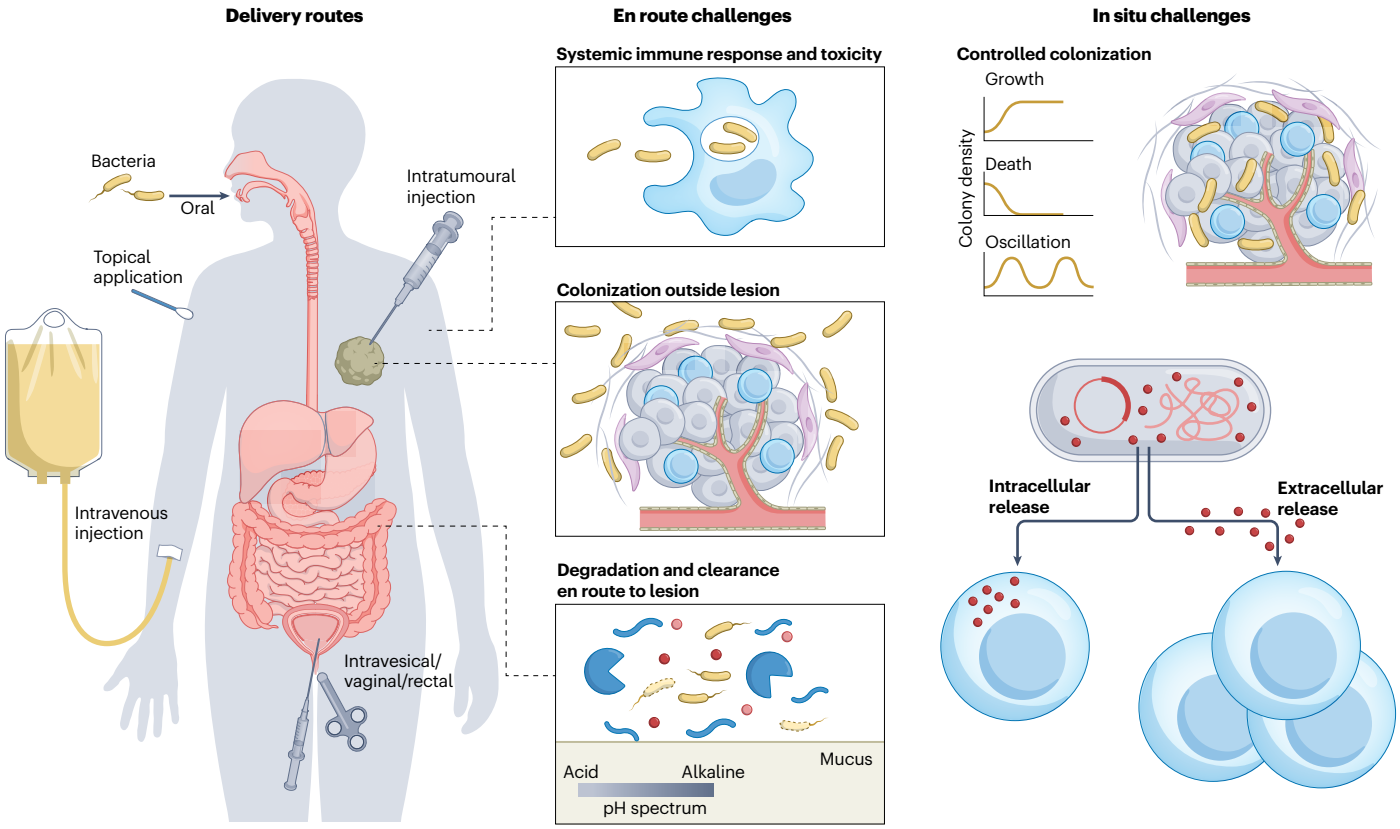

Fig. 1 | Challenges in delivery of bacterial therapy. Depending on the administration route, bacterial therapy faces different barriers to effective delivery. Intravenous injection of bacteria can lead to systemic immune response and toxicity owing to the presence of pathogen-associated molecular patterns. Intratumoural injection of bacteria can circumvent some challenges associated with systemic administration, but the proliferation of bacteria requires tight control of bacterial containment in the tumour to prevent leakiness into the bloodstream of surrounding tissues. Oral delivery can avoid most challenges from intravenous and intratumoural administration, but a harsh gastrointestinal environment necessitates strategies to avoid fast degradation and clearance to provide therapeutic effects. Once therapeutic bacteria are delivered to the target site, it is necessary to control the colonization of bacteria and transport the payload into the appropriate locations (such as bacterial cytoplasm, extracellular environment, host cell cytoplasm and nucleus) for therapeutic effect.

Surface properties can also affect the in vivo fate of therapeutic agents. Although nanoparticles can have a negative, neutral or positive charge depending on their composition, most bacteria are negatively charged. Surface charge affects electrostatic interactions with other ions, molecules and cells, which can ultimately alter clearance, toxicity and cellular uptake. The impact of these properties on nanoparticle delivery in vivo has been extensively studied[68–70]; however, the effect of surface charge on bacteria in vivo has not yet been well investigated. Some bacteria have evolved to envelope their outer surface with positively charged molecules, and the resulting change in surface charge can alter their interaction with the environment; for example, they may be able to adhere to charged surfaces, affecting the colonization of medical implants and causing infection[71]. Customizing the surface charge of bacteria can help elucidate its effect on their in vivo fate. Moreover, other surface properties, such as roughness, rigidity and hydrophobicity, may also impact the therapeutic functionality of bacteria.

Surface functionalization of nanoparticles can alter their targeting capability and responsiveness through physical and chemical modifications[72]. Physical modification involves non-covalent bonds, such as electrostatic and hydrophobic interactions, to immobilize ligands on nanoparticle surfaces. Chemical modification involves functionalizing the nanoparticle surface with a chemical moiety that can form a covalent bond with the ligand; often, a spacer such as polyethylene glycol is used to facilitate ligand–receptor interactions. The same strategies can be applied to engineer bacterial surfaces. Alternatively, genetic strategies are used for bacterial surface modification. These approaches may differ in their permanence as chemical coatings are typically performed in advance in vitro, whereas genetically programmed surface properties are controlled and passed down to progenies in situ. However, genetic approaches are limited by what is achievable through biosynthesis, whereas, in principle, chemical and physical approaches are limited by the current state of synthetic capabilities.

Delivery challenges

The dynamic nature and heterogeneity of biological barriers pose challenges to the delivery of any therapeutic. There is a dose-dependent balance between therapeutic effect and safety depending on the PK/PD of the drugs, which govern their clearance, toxicity, biodistribution and efficacy. The ability to sense and respond to environmental cues enables bacteria to autonomously modulate their properties, which may help them overcome these challenges. However, there are additional

considerations for using bacteria as therapeutics owing to their interactions with the human immune system (Fig. 1). Therefore, a deeper understanding of these challenges is needed for the rational design of optimally engineered bacterial therapies.

Oral delivery is the most common route of drug administration and has a high level of patient compliance. However, orally administered drugs must survive the harsh acidic and enzymatic environment in the stomach, rendering it difficult to deliver biologics such as proteins, peptides and nucleic acids[73,74]. The intestinal epithelium of the human gastrointestinal tract (GIT) is also lined with mucus and intestinal microbiota that act as a physical barrier to invading pathogens[75,76]. In between and beneath epithelial cells, immune cells are positioned to limit the leakage of commensal microbiota from the GIT into systemic circulation[77]. Housing around 10^{14} microorganisms[78], the human intestine serves as a niche for many bacteria by providing nutrients[79] and immune privilege resulting from interactions between native microbiota and the intestinal immune system[80]. A unique advantage of bacteria over nanoparticles for oral delivery is their capability to colonize the GIT as the long-term presence of bacteria could provide continuous therapeutic effects locally or systemically.

However, bacteria face similar challenges to those of nanoparticles to reach and colonize the GIT. Upon administration, extreme changes in pH throughout the GIT can diminish the viability of orally delivered bacteria[81,82]. Furthermore, orally delivered bacteria are met with colonization resistance by the indigenous microbiota and individual host features[83]. Therefore, effective colonization often requires the protection of bacteria from harsh acidic environments in addition to the resolution of colonization resistance from the endogenous gut microbiome. Although bacteria can be protected by taking advantage of tools developed for the oral delivery of other therapeutics (such as encapsulation), the main strategy to overcome colonization resistance has been limited to the administration of antibiotics to wipe out the indigenous microbiota, which could lead to dysbiosis and increases in opportunistic pathogens[84]. To address the transient colonization of the GIT typically seen with probiotics, native *E. coli* strains were isolated from murine and human hosts and verified to be genetically tractable for transgene delivery[85]. A native *E. coli* strain from the murine host was engineered to express bile salt hydrolase to influence host metabolism or IL-10 for an anti-inflammatory effect. The resulting strain exhibited lasting colonization in the gut of all treated mice ($n = 8$) for over 110 days in a non-sterile, low-barrier facility when introduced to a similar murine host via oral gavage[85]. Though this work advances our ability to stably colonize the host GIT using native microbiota, it is currently limited to *E. coli* as a bacterial scaffold. A personalized probiotic approach could solve interpersonal differences and pathology-induced changes in the micro-niches but such an individually tailored strategy requires substantial developmental cost and time. This challenge was addressed through a chemical approach whereby the primary amine groups on the surface of *E. coli* Nissle 1917 (EcN) were converted into thiols to form covalent bonding with disulfide-rich mucus[86]. This strategy was compatible with different strains of both Gram-negative and Gram-positive bacteria. The modified EcN achieved up to 170-fold higher attachment

Box 2

Safety and efficacy of bacterial therapy

An early effort to develop bacterial therapy focused on the attenuation of bacteria to improve safety. For example, the elimination of lethal α-toxin rendered *Clostridium novyi* non-pathogenic, which was used in clinical trials (NCT00358397, NCT01118819, NCT01924689 and NCT03435952) for the treatment of human and canine cancer[190–193]. Similarly, VNP20009, a *Salmonella typhimurium* (STm) strain used in clinical trials (NCT00004216, NCT00004988 and NCT00006254)[194], has a deletion of the *msbB* gene that is involved in the myristoylation of lipid A, which is essential in activating a Toll-like receptor 4 (TLR4)-mediated immune response[195]. In addition, VNP20009 has a deletion of the *purI* gene that introduces a purine auxotrophy to control growth. Preclinical studies of VNP20009 demonstrated improved safety with sustained tumour colonization and preserved therapeutic efficacy in mice[195,196]. However, in a clinical trial of patients with melanoma, intravenous administration of the strain at a maximum tolerated dose of 3×10^8 cfu m^{-2} showed bacterial colonization of tumour tissue in only one of six patients, and the colonization failed to show any efficacy[194]. Although the contradicting results between preclinical and clinical studies can be attributed to the differences between humans and mice, it also suggests that these attenuations may compromise the therapeutic efficacy and colonization of human tumours. For example, purine auxotrophy may affect the overall fitness of the strain, particularly in melanomas that are known to be low in purine synthesis[197], and be detrimental to tumour colonization. Instead, the antitumour effect of STm may be boosted by engaging a TLR4-mediated immune response. In another study, improved safety was observed upon knockout mutation of *rfaD* or *rfaG* genes, which are involved in lipopolysaccharide biosynthesis by STm in preclinical models, but these mutant strains were unable to achieve full therapeutic effect[198]. However, efficacy could be restored by conditional complementation of the deleted[198] genes. *Listeria monocytogenes* (Lm) is another type of intracellular pathogenic strain that has been attenuated and used for the treatment of cancer, mostly in the form of a cancer vaccine to expose the immune system to tumour-specific antigens. A commonly used Lm strain for such cancer vaccines has a weakened expression of the *prfA* gene, a 'master regulator' transcription factor for virulence genes, including that encoding listeriolysin O[199,200]. A complete deletion of *prfA* reduced the ability of Lm to escape from the vacuole, thus conferring inefficient antigen delivery to the cytoplasm for presentation by antigen-presenting cells, suggesting the importance of judicious attenuation that does not heavily compromise its efficacy. The development of next-generation bacterial therapies will benefit from adopting precision medicine approaches to balance safety and efficacy through application-specific and patient-specific modulation of immunogenicity using synthetic biology and nanomedicine with spatiotemporal control.

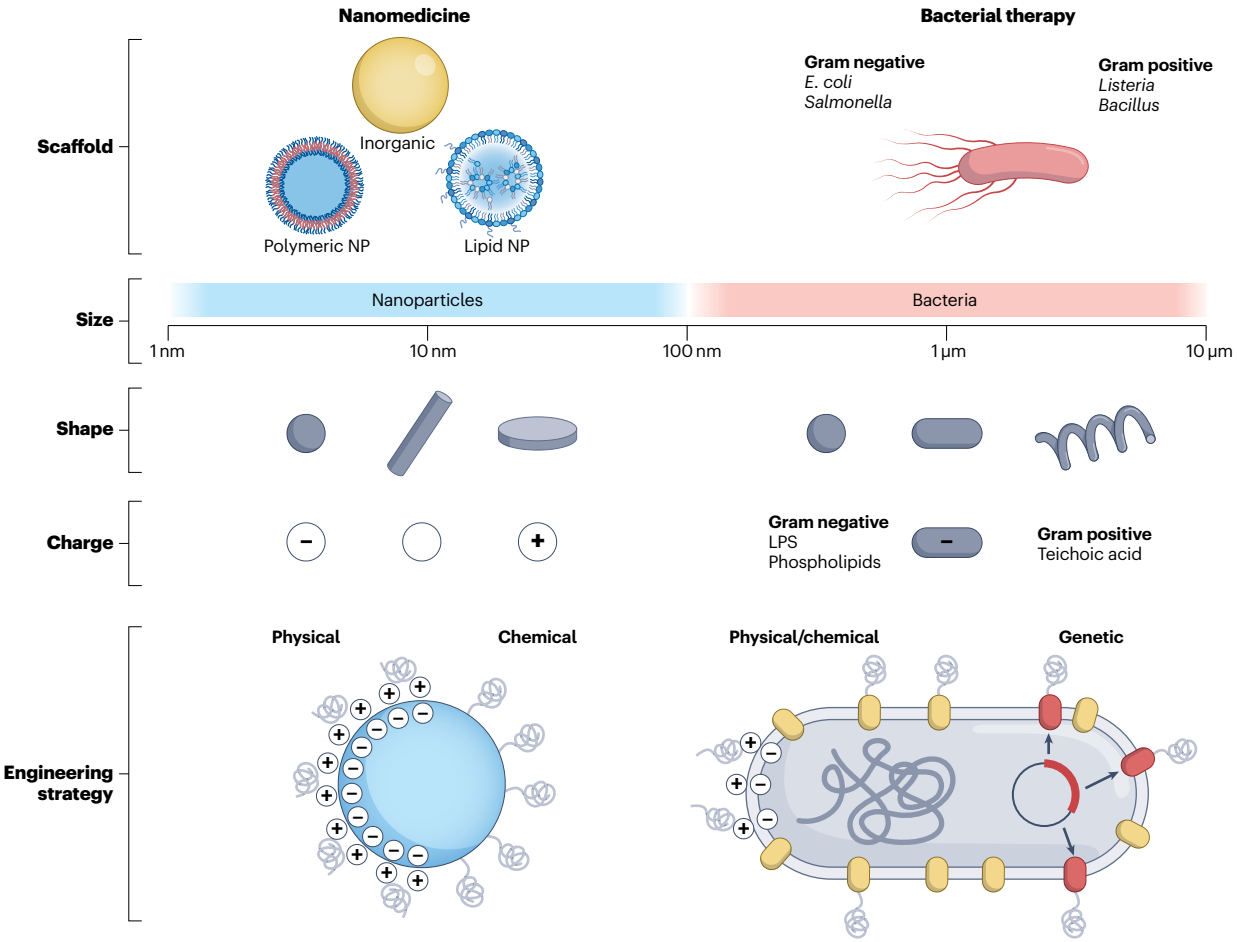

Fig. 2 | Comparison between nanomedicine and bacteria-based therapy. Design considerations, such as scaffold, size, shape, charge and engineering strategies, determine the fate of therapeutic agents in vivo. Here, some notable differences and similarities between nanomedicine and bacterial therapy are highlighted. LPS, lipopolysaccharides; NP, nanoparticle.

in mucin-rich jejunum compared to unmodified bacteria in mice but the fold difference decreased over time, likely caused by the loss of surface thiol groups through bacterial reproduction. Alternative and complementary strategies in the future could improve bacterial colonization while minimizing disruption to the native microbiome.

For applications where systemic delivery is necessary, such as bacterial colonization of tumours outside of GIT, oral administration requires that bacteria survive the digestive tract and translocate to the bloodstream. Though some orally ingested bacteria, such as *Bifidobacterium breve* UCC2003 and EcN, could translocate through gut epithelia to reach subcutaneous tumours[87] and liver metastases[88], respectively, it may be limited to specific bacterial scaffolds or target organs such as the liver that accumulate bacteria in circulation for clearance. Intravenous administration is an alternative route that can circumvent some of these challenges. For example, the vascular system provides access to a wide range of tumours, including primary and metastatic tumours that have undergone the angiogenic switch. Upon arrival, some bacteria can take advantage of the immunoprivileged tumour microenvironment (TME) to colonize tumours. Although tumour colonization is still not completely understood, colonization by bacteria is often associated with regions of necrosis, hypoxia and fewer numbers of immune cells, which results in stable bacterial numbers over time.

The two major hurdles for intravenous administration are rapid systemic clearance preventing colonization and associated toxicities. Bacteria are readily cleared from the circulation by the mononuclear phagocytic system composed of phagocytic macrophages, monocytes and dendritic cells mainly residing in the liver and spleen. Additionally, complement-binding in the blood can lead to the direct lysis of Gram-negative bacteria or assist in phagocytosis by immune cells, reducing the number of bacteria en route to tumour environments. Because rapid clearance of pathogens is important, bacteria are flagged for removal by recognizing PAMPs through pattern recognition receptors. The recognition of PAMPs by TLRs, a type of pattern recognition receptor, activates the secretion of inflammatory cytokines that can lead to a rapid onset of toxic inflammation and sepsis, depending on the scale and type of infection. Thus, unchecked bacterial colonization of healthy tissues or leakage of microorganisms from tumours could lead to infection and serious health risks. To enable safe intravenous delivery, bacterial species must be attenuated; several species from the genera *Salmonella*, *Listeria*, *Clostridium*, *Bifidobacterium* and *Escherichia*

have thus been genetically engineered to decrease immunogenicity and prevent unwanted replication. Although attenuation renders these bacteria safer, it may also diminish colonization and their therapeutic effect, suggesting that alternative strategies might be needed to improve safety and preserve efficacy (Box 2).

Local delivery is an alternative route that can circumvent the challenges of intravenous administration. For example, an intratumoural injection may overcome poor vascularization of the tumour tissue, such as in pancreatic adenocarcinomas[89], or physical barriers such as the blood–brain barrier that prevents the pharmacological treatment of most brain tumours[90]. Intravesical delivery, where bacteria are placed directly into the bladder through a catheter, is currently used clinically for BCG administration in patients with bladder cancer. Furthermore, topical delivery of microorganisms for skin applications is also being developed to elicit antitumour immunity or treat skin rashes[91,92]. Some limitations of local delivery approaches are that they may require invasive procedures and more complex image-guided techniques, depending on the tissue of interest. Moreover, leakage to surrounding tissues might affect the safety of bacterial injection as inflammation and immune response can vary, especially in an organ like the brain[93]. Besides, the containment of replicating bacteria is still necessary to minimize leakage and prevent possible infection and bacteraemia, even with local delivery.

Depending on the route of administration, bacterial biodistribution will differ based on the access to, clearance from and colonization of different tissues. Although oral delivery is generally regarded as safe in terms of limiting biodistribution to the GIT, intravenous injection increases the risk of non-specific tissue distribution. The main destinations of intravenously injected bacteria are the liver and spleen owing to their role in bacterial clearance through macrophage phagocytosis. However, bacteria that survive rapid clearance can reach other organs and increase the risk of infection. Because bacteria can replicate, even tighter control of biodistribution is necessary compared to that required for nanoparticles. Furthermore, colonized tissue serves as a reservoir for bacteria and contributes to long-term biodistribution. Ideally, therapeutic bacteria would be able to evade phagocytosis and survive in circulation long enough to reach the site of disease, while remaining sensitive to host defences in healthy tissues and minimizing off-target accumulation.

Regardless of how bacteria are delivered, another challenge of living drugs is adopting conventional pharmaceutical principles such as PK/PD to the development of bacterial therapy. Conventional PK/PD analysis cannot capture the complexity of bacterial therapy, and the presence of microbiomes in different tissues and organs (for example, gut) further complicates determination of the biodistribution of delivered bacteria. Furthermore, the replicating capability can make it challenging to establish the dose–response relationship as the initial dose may not represent the actual number of bacteria resulting in a therapeutic effect. One solution could be the use of non-replicating bacteria, such as auxotroph strains, that lack essential genes and cannot survive without certain metabolites only present during the manufacturing process. This strategy can also prevent unwanted replication in healthy tissues and resolve some safety concerns. However, it will also weaken the advantage of bacteria as living drugs that can self-replicate and amplify therapeutic effects. The pharmacokinetics of bacteria-based therapeutics have been modelled by measuring viable bacteria in different tissues to build simple pharmacokinetic models[94], but further investigation is needed to develop experimental techniques and models that fully capture the complexity of bacterial therapy.

Nanomedicine and bacterial therapy

Advancements in nanomedicine have enabled better delivery and therapeutic efficacy of nanoparticles, which have inspired novel bacterial engineering approaches for therapeutic applications (Fig. 3). This section covers recent developments at the interface of nanomedicine and bacterial therapy to develop next-generation therapeutics.

Nanomedicine-inspired bacterial systems

The human immune system has evolved to resist and tolerate bacterial colonization in response to interactions with pathogens, commensals and probiotics. The introduction of therapeutic bacteria in vivo is challenging owing to the tight regulation by the immune system and resident microorganisms that maintain homeostasis.

An effective engineering strategy to circumvent this issue in nanomedicine is to use a protective coating to camouflage the enclosed cargo from the immune system for prolonged circulation in blood. Similarly, EcN was wrapped with red blood cell membranes rich in self-antigens, such as CD47, to reduce engulfment by macrophages and lower the in vivo inflammatory response when administered intravenously[95]. The coating did not substantially affect the bioactivity of bacteria and increased the intensity and duration of bacterial

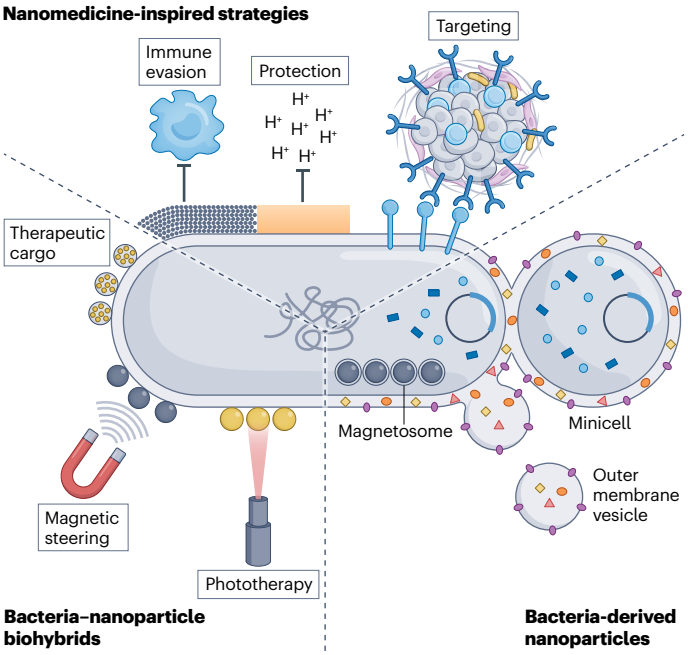

Fig. 3 | Interface between nanomedicine and bacterial therapy. Strategies from nanomedicine are adapted to engineer bacteria for immune evasion, protection from harsh environments and targeting of specific tissues. Camouflaging bacterial surfaces inhibits macrophage phagocytosis by hiding pathogen-associated molecular patterns. Encapsulation allows bacteria to resist acidic environments by providing physical barriers. Surface decoration of ligands against overexpressed receptors targets tumours and improves the colonization of bacteria. Nanoparticles are directly conjugated to the bacterial surface to synergize with bacteria. Delivery of drug-carrying nanoparticles can be improved by bacterial strategies to infiltrate mammalian cells. Magnetic nanoparticles allow magnetic manipulation of bacteria and in vivo guidance to target tissues. Gold nanoparticles act as agents for photothermal therapy. Bacteria can produce biological nanoparticles, such as magnetosomes, outer membrane vesicles and minicells, for medical applications.

luminescence during imaging of subcutaneous tumours in syngeneic mouse models of 4T1 breast and CT26 colorectal cancer. More advanced strategies in nanomedicine use responsive materials to dynamically modulate the surface properties depending on the environmental cues. Along these lines, a bacterial system that controls the surface expression of the bacterial capsule (a protective layer naturally found on the outer membrane of some bacteria) was developed[94]. The system was programmed to express the capsule in the presence of inducers, which enabled initial immune evasion when administered systemically, followed by fast clearance from off-target organs by immune cells through the shedding of the capsule. This strategy increased the maximum tolerated dose of EcN by ten times when injected intravenously in mice, which in turn improved therapeutic efficacy. Bacterial systems such as these highlight the possibility of achieving a delicate balance between safety and efficacy.

Another engineering strategy used in nanomedicine is encapsulation to protect nanoparticles from harsh in vivo environments such as low pH and enzymatic stress of the digestive tract. A similar strategy has also been applied to protect bacteria for oral delivery[96,97]. For example, a double-layer coating approach was used wherein EcN was first coated with a tannic acid layer, followed by coating with L100 Eudragit polymer[98]. As the encapsulated EcN exited the stomach, the L100 polymer dissolved to expose the mucoadhesive tannic acid layer for enhanced colonization of EcN in the small intestine. The resulting increase in colonization efficiency in turn improved the prevention and treatment efficacy of EcN against dextran sulfate sodium-induced colitis in mice. This example demonstrates an effective strategy to improve the therapeutic efficacy of bacteria by matching the progression of environmental changes along the delivery route.

Reflecting the success of decorating nanoparticles with targeting ligands, bacteria have been similarly functionalized with active targeting moieties to improve colonization at tumour sites. One approach is to display tumour-targeting ligands on the cell surface of bacteria, much like in tumour-targeting nanoparticles[99]. Conjugating aptamers on the bacterial surface increased tumour localization of intravenously injected VNP20009 and achieved up to fourfold higher tumour accumulations compared to unmodified bacteria in a 4T1 tumour-bearing mouse model by targeting nucleolin overexpression[100]. Furthermore, the increased localization translated into enhanced antitumour efficacy along with activation of immune responses inside the tumour. In addition to chemical modification, bacteria can be genetically engineered to change surface properties such as the expression of targeting ligands[12,101–103]. To target tumours overexpressing integrin αvβ3, VNP20009 was engineered to display the Arg-Gly-Asp peptide on the external loop of OmpA, leading to a >1,000-fold increase in colonization on αvβ3-positive tumours compared to that achieved by the control strain[104]. The increased colonization resulted in tumour regression and prolonged survival in mouse models of human breast cancer and melanoma.

Beyond modulating the interaction with the environment to improve delivery, nanoparticles have been engineered with multifunctionality to increase their efficacy through synergy between delivered therapeutics. This approach was translated for bacteria-mediated cancer immunotherapy by decorating EcN with polydopamine for photothermal therapy, with tumour antigens for the programming of dendritic cells and with anti-PD-1 antibodies for checkpoint blockade therapy[105]. The resulting system elicited potent immune responses and synergistically enhanced immunotherapy in mice with ovalbumin-overexpressing tumours. The versatility of polydopamine-coated bacteria as a multifunctional platform has been further leveraged to stimulate antiviral immunity[106] and enhanced photothermal therapy[107] by the functionalization of bacteria with a virus-specific antigen and photosensitizers, respectively.

Bacteria–nanoparticle biohybrid systems

Although nanoparticles can be imbued with functionalities hard to achieve in biological systems, bacteria can be engineered with complex behaviours not possible with current nanotechnology and molecular engineering strategies. Therefore, nanoparticles and bacteria have complementary properties that could synergize with one another when combined. Biohybrid systems consisting of bacteria and nanomaterials have been developed to leverage their complementary advantages for therapeutic applications.

Magnetotactic bacteria can be externally guided using magnetic fields in vivo by aligning the magnetosome, an organelle consisting of a chain of Fe_3O_4 nanoparticles used for geomagnetic navigation[108]. However, not all bacteria possess magnetosomes and it can be challenging to genetically engineer bacteria to express the organelle. Alternatively, magnetic nanoparticles can be conjugated on bacteria to artificially render them magnetotactic[109–111]. A bacterial biohybrid system capable of self-propulsion guided by magnetic field gradients was constructed using biotin–streptavidin interactions to couple *E. coli* MG1655 and magnetic nanoparticles[112]. Biotinylated nanoliposomes were able to decorate the bacteria in the presence of streptavidin and carry chemotherapeutics and photothermal agents for stimuli-responsive drug release. The system was able to navigate through the 3D collagen matrix mimicking the TME, reach tumour spheroids guided by a magnetic field and release anticancer drugs through near-infrared light activation. Although the magnetic field-driven biohybrid system is promising for drug delivery, its reliance on a directional magnetic field to guide bacterial movement has limitations such as poor scalability and the requirement for active positional feedback. Therefore, the clinical translation of magnetotactic bacteria requires alternative strategies to increase tumour infiltration of diffuse bacteria in the body once administered systemically. To overcome this limitation, magnetic torque was used as an alternative control scheme to increase tumour infiltration by magnetotactic bacteria, *Magnetospirillum magneticum* strain AMB-1. Instead of guiding the bacteria towards a specific direction or location using a directional magnetic field, the application of magnetic torque maximized surface exploration at the cell interface to improve tumour infiltration. Magnetic torque-driven bacteria were able to achieve a fourfold increase in translocation across the in vitro model of vascular endothelium and 21-fold higher colonization of tumour spheroids. A threefold higher accumulation of bacteria was observed in tumours on which magnetic torque was applied following intravenous injection of magnetotactic bacteria in a mouse model of breast cancer with subcutaneous MCF-7 tumours[113].

Bacteria can gain the unique properties of nanoparticles by forming biohybrid systems to potentiate their therapeutic efficacy[114,115] and acquire new functions[116–118]. For example, flagellated VNP20009 bacteria were decorated with cationic nanoparticles to absorb tumour antigens released from irradiated tumours[119]. By transporting tumour antigens to the tumour periphery, where active dendritic cells reside, the biohybrid system engaged adaptive immunity and induced systemic antitumour effects in multiple tumour models in mice when injected intratumourally following radiotherapy. Mice showing complete tumour regression were rechallenged through injection of tumour cells without apparent tumour engraftment,

showing that the treatment was indeed engaging adaptive immunity. The synergy was accomplished by cationic nanoparticles that acted as a backpack to collect negatively charged tumour antigens from the environment and flagellated bacteria that provided motility and acted as a potent adjuvant. In another study, spatiotemporal control of bacteria lysis for therapeutic release was achieved by coupling engineered bacteria with magnetic nanoparticles using glycoprotein-mediated click chemistry[120]. *E. coli* BL21 was programmed to express a bacteriophage lysis gene under the control of a heat-sensitive promoter, and the attached magnetic nanoparticles converted magnetic signals into heat and active navigation. When the bacteria constitutively expressed anti-CD47 nanobodies, the magnetically controlled lysis of bacteria upon intravenous injection elicited potent antitumour immunity in syngeneic and orthotopic mouse models of colorectal cancer. The magnetic nanoparticles acted as antennas to manipulate the engineered bacteria for cancer immunotherapy.

Many nanoparticles exert their therapeutic effects by delivering their cargo into the cytoplasm or other subcellular organelles such as the mitochondria or nucleus. However, most nanoparticles end up being trapped and degraded in intracellular vesicles (such as endosomes) before delivering their therapeutic cargoes. To overcome this challenge, the intracellular invasion capacity of *Listeria monocytogenes* was leveraged to deliver nanoparticles and their cargo into cells[121]. Bacteria were able to piggyback polystyrene nanoparticles coated with streptavidin by binding with biotinylated C11E9 antibodies that targeted the surface protein N-acetylmuramidase. Biotinylated plasmid DNAs were adsorbed on the nanoparticle surface and were delivered into the cytoplasm through the pore-forming capacity of listeriolysin O, reaching the nucleus to express the encoded genes. The resulting biohybrid system was able to transfect mouse cells to express the firefly luciferase genes in vivo when injected intraperitoneally, demonstrating its potential for effective intracellular delivery.

When administered systemically, nanoparticles are cleared rapidly through the kidney and/or mononuclear phagocytic system and may end up in off-target tissues, where they can damage healthy cells. Although nanoparticles can be engineered to evade clearance and actively target tumour cells to circumvent these problems, the lack of receptors on tumour cells orthogonal to those on healthy cells makes it difficult for nanoparticles to accumulate specifically in tumours. Despite the enormous effort devoted to the targeted delivery of nanoparticles for cancer applications, the median delivery efficiency of nanoparticles is 0.7%[122]. Meanwhile, some bacteria can effectively target the hallmarks of the TME (for example, acidic, hypoxic, immune privileged) instead of specific target receptors to reach and colonize tumours[123,124] and act as a carrier to deliver nanoparticles[125–127]. To harness this potential for nanoparticle delivery, drug-loaded nanoliposomes were attached to magneto-aerotactic bacteria *Magnetococcus marinus* strain MC-1, which can naturally migrate to and maintain position at the preferred low oxygen environment[128]. To further guide bacteria towards tumours, this biohybrid system was externally controlled using a magnetic field aligning the magnetosome. Each bacterium could carry approximately 70 nanoliposomes, and up to 55% of bacteria could reach hypoxic regions of HCT116 colorectal xenografts in immunodeficient beige mice when injected near and magnetically guided towards tumours. The study demonstrated that bacteria-mediated tumour targeting can be leveraged to increase the delivery efficiency of existing nanocarriers into the TME for cancer diagnostics and therapies.

Bacteria-derived nanoparticles

The replicative property of bacteria can be advantageous via in situ amplification of the system but also deleterious through uncontrolled growth that can lead to off-target toxicity. Moreover, the larger size of bacteria compared to nanoparticles poses a physical barrier to efficient delivery into target tissues. Instead of using live bacteria, nanoparticles can be synthesized using bacteria either as a factory or as a base material. Decreasing the complexity results in some loss of function but also in a more predictable system for in vivo applications, and these nanoparticles are often easier to store and handle compared to live bacteria.

Various metallic nanoparticles have been synthesized in bacteria either naturally or through genetic engineering[129–131]. In particular, magnetosomes can be extracted as bacteria-derived iron oxide nanoparticles enveloped in bacterial membranes. Depending on the parent bacteria, the resulting nanoparticles possess different shapes and sizes, typically 35–120 nm in diameter[132]. The isolated magnetosomes can be further functionalized through the modification of proteins and lipids with targeting ligands and drugs for diagnostic and therapeutic applications. For example, magnetosomes were extracted from *Magnetospirillum magneticum* AMB-1 and decorated with gold nanoparticles through in situ growth for cancer therapy[133]. The resulting construct accumulated in tumours when guided by magnetic fields and acted as contrast agents for photoacoustic imaging and magnetic resonance imaging. For therapy, gold nanoparticles acted as starving agents through glucose consumption and as photosensitizers for near-infrared laser irradiation. Furthermore, the acidic condition of the TME triggered the release Fe^{2+} iron from the magnetic nanoparticles, which led to the subsequent production of reactive oxygen species through Fenton reactions with H_2O_2 abundant in the TME, resulting in chemodynamic therapy. The multimodal theranostic nanoparticles showed efficacy against the 4T1 mouse breast cancer model and multiple human xenograft models, including patient-derived xenografts. Magnetosomes can be easily produced in bacteria and be chemically or genetically modified to have multiple diagnostic and therapeutic functions, making them promising bacteria-derived nanoparticles for biomedical applications.

Outer membrane vesicles (OMV) are bacteria-derived nanoparticles naturally produced by Gram-negative bacteria. OMVs vary in size and composition, carrying membrane components and the periplasmic content of their parent bacteria. The immunomodulatory nature of OMVs garnered interest in the development of vaccines, adjuvants[134,135] and immunotherapeutics[136]. For example, OMVs from *Akkermansia muciniphila* in the GIT assist in restoring gut homeostasis and regulating mucosal immune responses[137]. Moreover, OMVs have been explored as drug-delivery vehicles through genetic engineering of parent bacteria and direct modification of OMVs. For example, bacteria have been engineered to produce OMVs with targeting capability and nucleic acid cargo loads as therapeutics[138]. To target cancer cells overexpressing HER2 receptors, an anti-HER2 affibody was fused to the C-terminus of ClyA cytotoxin to display recombinant proteins on the outer surface of bacteria and their OMVs. The OMVs with targeting capability were further loaded with short interfering RNA cargo through electroporation to silence kinesin spindle protein overexpressed in rapidly proliferating cells such as cancer cells. The engineered OMVs were injected intravenously into mice bearing HCC-1954 xenografts and showed targeted delivery to tumours, resulting in the inhibition of tumour growth with no evidence of non-specific side effects. Overall, OMVs represent a flexible platform amenable to genetic and chemical modifications, and their diversity and natural immunomodulatory properties offer

Box 3

Translational considerations

Multiple clinical trials are currently ongoing for engineered microbial therapeutics (Table 1). Although early data indicate some promising results, clinical approval of this new class of therapeutics must fulfil several criteria. For example, the transmission of engineered genetic components to the surrounding environment needs to be prevented. This includes antibiotic cassettes and plasmids that are commonly used to ease genetic modification and maintenance during laboratory development. However, it is also necessary to ensure plasmid stability for the function of bacteria-based therapeutics with strategies such as toxin–antitoxin systems[201] and synthetic auxotrophic systems[202]. Live biotherapeutic products can have genomically integrated payloads to circumvent the need for plasmids and antibiotic cassettes, but the long-term effect of such modifications needs to be validated for safety and efficacy. Furthermore, it is still necessary to ensure the genomic stability of introduced genetic elements[203]. As there is no existing example of engineered bacteria for direct injections into the body in the clinical setting, similar modalities can provide useful guiding principles for regulatory approval. This includes genetically engineered microbial agents, such as oncolytic viruses, and mammalian cellular therapies such as chimeric antigen receptor (CAR) T cells and stem cells. Moreover, costs involved with the manufacturing and administration at scale will need to be considered, including payments for health-care providers and insurance companies. As bacterial therapy enters late-stage clinical trials, implementation of these approaches will become a key determinant of success.

Integration of therapeutic bacteria with the existing standard of care will become increasingly important as researchers start to consider the implementation of this technology in current medical practice. In cancer, the interaction of bacteria with other modalities, such as chemotherapy, immunotherapy, radiation and surgery, will be crucial moving forward. For example, surgical resection may disrupt hypoxic and necrotic regions of solid tumours, which serve as colonization niches for several therapeutic bacteria. Patients treated with chemotherapy may be immunocompromised and receive antibiotic treatment, which will likely impact the efficacy and safety of bacteria. Although the interaction of bacterial therapies with existing interventions may pose challenges, bacteria can also synergize with chemotherapy, immunotherapy and radiotherapy in some cases[204–207]. As emerging therapy intersects with other modalities, more innovative combinations are expected to arise that address critical challenges for disease treatment.

Lastly, it is important to examine what has contributed to the success of FDA-approved bacterial therapies such as Vaxchora and Bacillus Calmette–Guérin (BCG). These therapies, for the most part, leverage their interaction with the immune system through vaccination or immunotherapy and involve attenuated live bacteria that are unable to colonize the patient over an extended period. Therefore, judicious and strategic use of their interactions will be one of the key ingredients to future success. Furthermore, the current administration methods (that is, oral, intravesical and rectal) keep the bacteria locally, which increases their safety. Even oral administration, which is considered systemic delivery for small-molecule drugs absorbed into the circulatory system from the digestive tract, should be considered local for bacteria, which face a substantially higher barrier to escape from the digestive tract. Therefore, in the near future, we expect that FDA-approved bacterial therapies will be limited to those delivered locally. However, this challenge also points to the enormous opportunity for the field to develop better technologies to investigate and enable systemic delivery (for example, intravenous) of live bacterial therapies.

the potential to develop bacteria-derived nanoparticles for biomedical applications.

Rather than relying on natural isolates from bacteria, bacteria can be modified to artificially produce nanoparticles. Minicells can be produced from *E. coli* by mutating genes located in the minicell locus, *minB*, which causes aberrant cell division resulting in achromosomal cells[139]. Much like OMVs, minicells can be engineered to target specific tissues and carry payloads[140]. Minicells also contain cytoplasmic material from parent bacteria, which can include plasmids and can be used to carry out functions programmed via gene circuits, as shown with the programmable detection of small molecules using minicells derived from *E. coli*[141]. Parent bacteria were engineered with different biosensing circuits to produce green fluorescent protein (GFP) in the presence of the target molecule. When minicells were produced and purified to remove their parent bacteria, the presence of target molecules was able to induce the production of GFP in vitro. In another study, parent bacteria were engineered to express nanobodies on the outer membrane to target cancer cells and convert salicylate into catechol for anticancer therapy through an inducible gene circuit[142]. Minicells derived from the engineered bacteria specifically bound and killed Caco2 colorectal cancer cells in vitro. As a compromise between bacteria and nanoparticles, minicells could serve as genetically engineerable 'smart bioparticles' that can be programmed with more complex behaviours than nanoparticles constructed using bottom-up approaches[143,144] without the risks imposed by the use of replicating live bacteria.

Phage particles are another type of nanoparticle that is produced by bacteria. Phages are viruses that naturally infect bacteria and rely on bacterial machinery for their replication. Phages have become an important tool for biotechnology in the past decades, highlighted by the 2018 Nobel Prize in Chemistry recognizing phage display of peptides and antibodies[145]. These genetically encoded nanoparticles are composed of proteins and nucleic acids and can thus be used to deliver protein-based and nucleic acid-based therapeutics. Their natural ability to infect bacteria has been leveraged for antibiotic applications over the past century[146,147], but they are only recently being explored as vaccines[148] and drug-delivery vehicles[149,150]. For example, hybrid adeno-associated virus phage particles from M13 filamentous phage were developed for gene delivery applications[151].

The adeno-associated virus phage particles were engineered with targeting ligands for improved uptake, protective motifs against degradation and inverted terminal repeats from adeno-associated viruses for improved transgene expression in the target cell. Another class of bacteria-derived nanoparticles closely related to phage particles are contractile injection systems (CISs) produced by some bacteria to deliver payload proteins into eukaryotic cells using phage tail-like nanomachines. A programmable protein delivery system was developed by engineering CIS from *Photorhabdus asymbiotica* to expand its target, including human and murine cells, and to deliver various non-native protein therapeutics such as Cas9, base editors and toxins[152]. Proteins were delivered in vivo by intracranially injecting CIS loaded with Cre in mice, which resulted in the activation of loxP-tdTomato in neurons without notable toxicity. The genetically encoded particles developed from phages and CISs are highly programmable and represent a versatile class of bacteria-derived nanoparticles for protein and gene therapy.

Outlook

Advances in microbial synthetic biology in the past two decades have allowed bioengineers to design and programme bacteria with unprecedented complexity in the test tube. These capabilities have transformed engineered bacteria into promising living drugs in preclinical studies. Although there have been several clinical trials with bacteria-based therapeutics to date, only a few have progressed to advanced trials and FDA approvals (Box 3 and Table 1). In general, many drugs fail in clinical trials owing to differences between preclinical models and human patients, which result in inadequate efficacy and excessive toxicity[153]. The same challenge applies to bacterial therapy and must be overcome to achieve the key milestone of demonstrating effective therapy using engineered bacteria in the clinic.

This Review has revealed that approaches to integrate nanomedicine and bacterial therapy vary widely and that improving the delivery of bacteria varies according to the required applications and therapeutic agents. To accurately determine what is needed to improve delivery, a general framework for the study of the PK/PD of bacterial therapies is necessary. Other living drugs, such as CAR T cells, would serve as a good starting point to build this framework[154]. Furthermore, in vivo imaging of bacteria is necessary to obtain real-time biodistribution data. Although optical imaging is useful for small animal models[155], establishing pharmacokinetics in human patients will require molecular imaging techniques with superior deep tissue performance, spatial resolution and sensitivity[156,157]. At the same time, the characterization of bacteria in vivo will be necessary to verify the stability of genetic elements[158] and transcriptomes[159] and to understand their mechanisms of action.

As delivered therapeutics encounter different biological barriers en route, dynamic modulation of bacterial behaviour, whether for oral delivery to the GIT or intravenous delivery to the tumour, is essential to ensure therapeutic safety and efficacy. The chemical diversity of nanotechnology and complex sense-and-respond genetic circuits afforded by synthetic biology are expected to complement each other and result in more sophisticated surface engineering strategies for therapeutic bacteria that meet the translational need. The target organ may be difficult to reach via diffusion, for example, owing to low vascularization and high interstitial pressure. Engineering of bacterial taxis mechanisms using biohybrid systems could augment bacterial motility towards different diseased sites in a precise manner[160–162]. Once at the destination, the dynamic presentation of binding motifs[98] and biosensor-driven tropism[163] can alter the biodistribution of bacteria and minimize the dose required for therapeutic effect.

Advances in the bioconjugation of nanoparticles to bacteria should allow industrial-level production of these biohybrid systems and increase the possible repertoire of combinations to construct multifunctional platforms. By combining bacteria and nanomaterials, these two platforms can have complementary properties to improve their intended functions or even create new functionalities that are not possible alone. However, such combinations must be compatible physically, chemically and biologically. For example, specific nanoparticles with desired properties might also have antibacterial properties

Table 1 | Clinical trials of selected bacterial therapies

Phase	Disease	Treatment	Route	Significance	Identifier/Refs.
I (completed)	Cancer (advanced or metastatic)	VNP20009 (engineered *Salmonella enterica* subsp. *enterica* serovar Typhimurium)	IV	*S.* Typhimurium is genetically engineered to delete *purI*, *msbB* and *xyl*	NCT00004988
I (ongoing)	Glioblastoma multiforme	EGFR(V)-EDV-Dox (engineered bacterial minicell)	IV	Bacterial minicell derived from *S.* Typhimurium minCDE-strain is engineered to target EGFR and carry doxorubicin	NCT02766699
I/II (suspended)	Solid tumours (advanced and/or metastatic)	APS001F (engineered *Bifidobacterium longum*) in combination with flucytosine and maltose	IV	*B. longum* is genetically engineered to produce cytosine deaminase	NCT01562626
I/II (discontinued)	Familial adenomatous polyposis	CEQ508 (engineered *Escherichia coli*)	Oral	An attenuated strain (undisclosed) of *E. coli* is genetically engineered to deliver β-catenin short-hairpin RNA	171
II (recruiting)	Metastatic pancreatic cancer	Saltikva (engineered *S.* Typhimurium) in combination with either FOLFIRINOX or gemcitabine/paclitaxel	Oral	An attenuated strain (undisclosed) of *S.* Typhimurium is genetically engineered to express IL-2	NCT04589234
III (recruiting)	Phenylketonuria	SYNB1934 (engineered *E. coli* Nissle 1917)	Oral	*E. coli* Nissle 1917 is genetically engineered to metabolize L-phenylalanine	NCT05764239

IV, intravenous; Dox, doxorubicin; EDV, EnGeneIC delivery vehicle; FOLFIRINOX, folinic acid, 5-fluorouracil, irinotecan and oxaliplatin.

Box 4

Manufacturing considerations

Living cell therapy demands rigorous biomanufacturing practices given the inherent heterogeneity and proliferative nature of the cells. Thus, stringent guidelines such as Good Manufacturing Practices have been set for existing cell therapies such as chimeric antigen receptor (CAR) T cells. For bacteria, industrial-scale manufacturing is common for biomolecule production and probiotic products, which provides a useful resource for ensuring quality and quantity. Genetic instability, either in plasmid or genome, is a major challenge for all genetically engineered living drugs when scaling up because the introduction of heterologous biological circuits can create negative selection pressure. Efficient growth of culture with reproducible genetic stability can be achieved by additional synthetic biology approaches geared towards manufacturing processes such as tolerance engineering, growth-coupled production and growth-decoupled production[208]. For example, bacteria can be engineered to activate therapeutic circuits in hypoxic conditions such as the mammalian gut using an anaerobic inducible promoter to control therapeutic production with oxygen concentration during the production stage[209]. In addition, a biosensor-based diagnostic system will be useful to evaluate the fidelity and performance of genetic circuits during the scale-up process[210].

An alternative to developing scale-up manufacturing methods for bacteria-based therapeutics involves in situ engineering of native microbiomes, which could overcome some manufacturing challenges and reduce therapeutic costs. For example, an M13 phage was engineered to deliver an exogenous CRISPR–Cas9 system to *Escherichia coli* within the mouse gastrointestinal tract to deplete the target strain during competitive colonization or enable genomic deletion of a target gene[211]. This strategy resembles voretigene neparvovec, marketed as Luxturna, which is the first FDA-approved gene replacement therapy against vision loss in the USA and in the European Union[212,213]. Though the scale-up challenge still exists for phage-based therapies, their simplicity compared to their bacterial counterparts lowers manufacturing hurdles and costs. Moreover, such a strategy does not require colonization of bacteria and long-term residence in the gut. However, it should also be noted that new challenges, such as bacterial resistance and biocontainment of infectious phages, must be addressed.

and would diminish the function of the coupled bacteria. Therefore, increasing the compatibility between the two platforms would expand the design space of such biohybrid systems.

Most studies involving biohybrid systems thus far have used pre-formulated complexes of nanoparticles and bacteria prior to administration. Advances in systems biology have shown the complexity of biological systems arising from dynamic interactions and multifaceted networks among individual cells[164]. To mimic such complexity, systems nanotechnology has also emerged to increase the functionality of individual nanoparticles[164,165]. This approach could also fit bacterial biohybrid systems owing to the versatility of nanoparticles and bacteria. For example, nanoparticles can be programmed to separate from extracellular bacteria at the hypoxic TME in response to low pH using acid-labile linkers for the internalization of nanoparticles by tumour cells[166]. A carefully designed set of rules that govern interactions between bacteria and nanoparticles would allow the emergence of complex behaviour to render biohybrid systems more robust and effective as therapeutics.

Bacteria-derived nanoparticles preserve the unique properties of bacteria without the risks associated with replicative, living drugs. These nanoparticles can be synthesized and purified to improve safety, although some loss of function is expected. Alternatively, OMVs presenting tumour antigens can be produced in the gut for cancer vaccine application in situ[167]: engineered *E. coli* TOP10 cells were ingested orally and delivered antigen-presenting OMVs that can cross the intestinal epithelium to stimulate dendritic cells to inhibit tumour growth and immunize against re-challenge in mouse models. This in situ production of bacteria-derived nanoparticles provides another strategy to deliver a payload from colonizing bacteria.

Although the main focus of this Review was bacteria-based therapeutics, another exciting avenue at the intersection of nanomedicine and synthetic biology is the use of bacteria as living diagnostics. The sense-and-respond genetic circuits enabled by synthetic biology can expand to diagnostic applications by producing reporter genes in response to biomarker detection[168]. For example, applying signal digitization and amplification to build 'bactosensors' allowed the detection of pathological levels of glucose in urine samples collected from patients with diabetes[168,169]. Going beyond the synthetic biology-enabled bacterial diagnostics, engineered bacteria and miniaturized electronic luminescence detectors were integrated to create ingestible biohybrid systems that can communicate with an external device in situ[170]. As bacteria are limited to producing biosynthetic molecules, further integration of nanomedicine is expected to increase the repertoire of biomarkers and reporter signals to create functional biohybrid diagnostics.

Bacterial therapies will face considerable challenges for clinical development and regulatory approval owing to the lack of predicate products and the potential stigma around 'bugs as drugs'. It will be necessary to establish patient acceptance through both outreach and continued technological advancement to satisfy societal concerns. Although their medical use is likely to be accepted for the treatment of life-threatening conditions, utmost care must be taken not to set poor precedents that can stump growth of the field. In addition, manufacturing challenges will have to be overcome for successful translation into the market (Box 4). The convergence of synthetic biology and nanomedicine will contribute to the development of better delivery strategies to ensure safety and efficacy and fuel the clinical translation of bacteria-based therapies.

Citation diversity statement

We acknowledge that papers authored by scholars from minoritized groups are systematically under-cited. Here, we have made every attempt to reference relevant papers in a manner that is equitable in terms of racial, ethnic, gender and geographical representation.

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