Title

Bacteria-Inspired Nanomedicine

Journal

ACS Applied Bio Materials, 4(5)

ISSN

2576-6422

Authors

Holay, Maya
Guo, Zhongyuan
Pihl, Jessica
et al.

Publication Date

2021-05-17

DOI

10.1021/acsabm.0c01072

Peer reviewed

HHS Public Access

Author manuscript

ACS Appl Bio Mater. Author manuscript; available in PMC 2022 May 17.

Published in final edited form as:

ACS Appl Bio Mater. 2021 May 17; 4(5): 3830–3848. doi:10.1021/acsabm.0c01072.

Bacteria-Inspired Nanomedicine

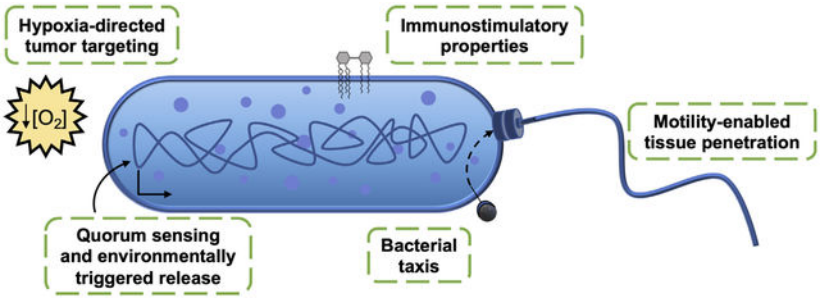
Maya Holay, **Zhongyuan Guo**, **Jessica Pihl**, **Jiyoung Heo**, **Joon Ho Park**, **Ronnie H. Fang***, **Liangfang Zhang***

Department of NanoEngineering, Chemical Engineering Program, and Moores Cancer Center, University of California San Diego, La Jolla, CA 92093, USA.

Abstract

The natural world has provided a host of materials and inspiration for the field of nanomedicine. By taking design cues from naturally occurring systems, the nanoengineering of advanced biomimetic platforms has significantly accelerated over the past decade. In particular, the biomimicry of bacteria, with their motility, taxis, immunomodulation, and overall dynamic host interactions, has elicited substantial interest and opened up exciting avenues of research. More recently, advancements in genetic engineering have given way to more complex and elegant systems with tunable control characteristics. Furthermore, bacterial derivatives such as membrane ghosts, extracellular vesicles, spores, and toxins have proven advantageous for use in nanotherapeutic applications, as they preserve many of the features from the original bacteria while also offering distinct advantages. Overall, bacteria-inspired nanomedicines can be employed in a range of therapeutic settings, from payload delivery to immunotherapy, and have proven successful in combatting both cancer and infectious disease.

Graphical Abstract



Keywords

Nanomedicine; biomimetic; therapeutic bacteria; drug delivery; immune modulation

*Corresponding authors: rhfang@ucsd.edu and zhang@ucsd.edu.

The authors declare no competing financial interest.

1. INTRODUCTION

In 1891, William Coley reported the curious observation that injection of *Streptococcus pyogenes* and *Bacillus prodigiosus*, later coined as 'Coley's toxins,' into cancer patients caused tumor regression.[1, 2] Ever since this discovery, researchers have had a growing interest in leveraging bacteria and their properties for medical advancements. Certain strains of bacteria are particularly suited for cancer therapy, as their anaerobic properties restrict their growth to the hypoxic tumor microenvironment, and their immune-simulating properties combined with their inherent toxicity enhance the body's antitumor response.[3] Successful translation of this strategy came in the form of Bacillis Calmette-Guérin (BCG), a live attenuated obligate anaerobic strain of *Mycobacterium bovis* that is used in the intravesical treatment of bladder cancer.[4] With further advancements in nanotechnology and genetic engineering, the complexity and application potential for therapeutic bacteria has grown significantly. Bacteria-based therapeutics have expanded to a variety of non-cancerous diseases, including infection, diabetes mellitus, and inflammatory bowel disease.[5] Since bacteria interact with a wide range of processes in the human body, it is worthwhile to carefully consider both their cooperative and destructive impacts and posit how these interactions may be leveraged for therapeutically beneficial outcomes.

It is thought that many of the first landmark clinical trials evaluating administration of *Salmonella* Typhimurium to combat solid tumors failed in part because the required attenuation strategies led to poor tumor colonization and lack of immune stimulation.[6] Certainly, one of the ongoing challenges in bacteria-based therapeutics is the balance between attenuation for safety considerations and maintenance of active targeting and immunogenicity. While bacteria were traditionally attenuated using chemical mutagenesis and serial *in vitro* passages, attenuation strategies have become more elegant with advancements in genetic engineering. Strains can be genetically altered to remove expression of virulence factors and aromatic amino acid synthesis regulators, or alternatively genes may be put under a nutrient-dependent or environmentally triggered promotor that can be carefully controlled.[7] Furthermore, various nanoscale derivatives of bacteria, such as membrane ghosts, extracellular vesicles, spores, and secreted proteins, oftentimes require little to no attenuation for safe administration[8] and present a promising direction for further advancement.

There are many ways in which bacteria and their nanoscale derivatives can be engineered for biomedical applications (Figure 1). For example, quorum sensing involves the self-production of chemical signals that can control the transcription of certain genes in the host bacteria when they reach a threshold concentration. This process can be utilized to control the production of a biomolecule of interest, including drug molecules and antibodies.[9] Advantages of employing this type of natural circuit include abundant and continuous payload production, as well as prevention of bacterial overgrowth.[10] Along with self-production of signaling molecules, bacteria can also be readily engineered for environmentally triggered payload release. Examples include arabinose-induced production of α-hemolysin[11] and hypoxia-promoted delivery of tumor necrosis factor (TNF)-related apoptosis-inducing ligand to tumor tissue.[12]

Certain bacteria, which often have at least one dimension on the nanoscale, also demonstrate taxis and swimming behavior that can improve targeting and accessibility of a nanotherapeutic to a site of interest. Flagella-propelled motility allows bacteria to swim deep into tissue, differentiating live bacteria from traditional therapeutics that can only passively diffuse and often fail to effectively penetrate tissue.[13] Bacterial taxis, defined simply, is the bias of movement towards or away from a certain stimulus, such as light, nutrients, or magnetic forces.[14] Coupled with bacterial motility and chemosensory pathways, taxis provides a mechanism for active targeting. This behavior has proven beneficial in both natural settings, where whole bacteria use taxis to accumulate in a specific tissue,[15] and synthetic settings in which it is modeled to improve delivery and accessibility of nanomedicine to its targets.[16]

Hypoxia provides the most prevalent tumor-targeting mechanism for bacteria. Due to the anaerobic growth conditions of many bacteria, proliferation can be limited to the hypoxic tumor microenvironment and excluded from healthy tissues.[3] Interestingly, in addition to tumor tropism, bacteria such as *Listeria* can infect antigen-presenting cells (APCs) and myeloid-derived suppressor cells in a sort of “immune cell hijacking” that helps them avoid clearance. Due to the accumulation of these immune cells in tumor tissue, *Listeria* can be selectively delivered to the tumor site using this mechanism.[3]

In the field of immunotherapy, bacteria can act as potent immunostimulatory agents and targeted delivery vehicles. Bacteria can directly lyse tumor cells, generating an abundance of tumor antigens that may accelerate an immune response. Further, bacteria-derived factors, including pathogen-associated molecular patterns (PAMPs), can initiate proinflammatory cytokine secretion that enhances immune cell recruitment.[3] Various bacteria components, including flagellin,[17] lipopolysaccharide (LPS),[18] and exotoxins,[19] can also act as potent adjuvants for nanovaccine formulations.

Informed by these design principles, this review explores the extensive use of bacteria and bacteria-inspired nanomaterials in payload delivery and immune modulation applications.

2. PAYLOAD DELIVERY

To maximize clinical benefit, therapeutic payloads should be capable of selectively targeting the tissue of interest. Therapeutics, nanoparticles in particular, may be conjugated with antibodies, aptamers, peptides, and other targeting moieties to help improve delivery;[20–23] however, these conjugated formulations can still face significant penetration and retention challenges at the organ, tissue, or cell level. Beyond reaching the target tissue, successful therapeutics should have tunable payload release to maintain efficacy over the required time period without generating systemic toxicity. Controlled release can be achieved through responsiveness to various triggers. Internal stimuli, such as pH changes in the endosome or intracellular stress, can induce drug release once the payload has been taken up by the cell of interest. External stimuli such as temperature, ultrasound, magnetic forces, or local nutrient conditions can help tune the release of the payload into the surrounding microenvironment.[24, 25] Bacteria, with their inherent tumor-targeting properties, self-propulsion, and taxis behavior, can act as delivery vehicles to a variety of tissues with tunable payload release

kinetics.[3] While bacteria-related safety issues represent an ongoing concern, they may be mitigated by the use of biocompatible carriers, genetic engineering strategies to reduce immunogenicity and toxicity, and bacterial subunits or synthetic mimics with inherently lower risk.[5]

2.1 Live Bacteria

The use of nanoparticle-carrying bacteria for payload delivery was introduced a decade ago, where it was demonstrated that the approach could be used for DNA-based model drugs.[26] Consequently, bacteria have begun to be exploited as microbial actuators instead of mere vectors for the delivery of therapeutics.[27] Due to engineering advances, the biological functions of microorganisms can be readily modified, allowing for advanced customization of therapeutic activity as well as spatiotemporal control.[27] In particular, live bacteria offer selective colonization and targeting, controlled taxis behavior, and prodrug delivery capabilities.

2.1.1 Selective colonization—By harnessing bacterial tumor-targeting properties, researchers have created whole bacteria-assisted targeted delivery systems for cancer therapy, imaging, and diagnosis.[3] For example, the facultative anaerobic bacteria *Salmonella enterica* serovar Typhimurium VNP20009 has hypoxia-mediated targeting capabilities and has been extensively studied for cancer treatment.[28] As an approach to enhancing antitumor efficacy, researchers have explored the combination of VNP20009 with photothermal agents.[29] Interestingly, photothermal treatment not only effectively lyses tumor cells, but such cell lysis also generates nutrients that further attract bacteria to the tumor area, resulting in enhanced therapeutic efficacy.[30, 31] In one instance, polydopamine was coated onto VNP20009 *via* oxidation and self-polymerization, thereby allowing for selective delivery of the photothermal agent to the tumor hypoxic region *in vivo*.[29] Despite its significant tumor-targeting ability, the therapeutic efficacy of *Salmonella* Typhimurium YB1 in large solid tumors is still limited because the bacteria exclusively accumulate in the hypoxic region while leaving well-oxygenated regions undestroyed.[32, 33] In one study, indocyanine green (ICG)-loaded nanoparticles were covalently linked to YB1 (Figure 2).[34] After colonization of the nanoparticle-modified YB1 in the tumor hypoxic region, the ICG payload was irradiated with near infrared (NIR) light, thereby destroying the surrounding oxygenated tumor tissue. The photothermal tumor lysis generated bacteria-attracting nutrients, which mediated further penetration of bacteria into the tumor tissue. Compared to a control group without photothermal therapy, the engineered bacteria paired with laser irradiation showed a 14-fold enhanced accumulation in the tumor tissue and increased tumor temperatures, thus blocking tumor growth.

Oral delivery is among the most commonly used drug administration routes.[35] The development of oral therapeutic peptide and nucleic acid formulations remains a challenge due to acidic destruction and enzymatic degradation in the gastrointestinal tract, as well as poor drug penetration across the intestinal membrane.[36] It was recently reported that live bacteria can protect therapeutic cargoes against degradation in the stomach, thus facilitating the oral delivery of proteinic drugs or nanoparticles.[26, 37, 38] As an example, *Escherichia coli* MG1655 was genetically engineered to express a therapeutic protein, TNF-α, in response to

thermal stimulation, and the bacteria were further decorated with gold nanoparticles by enzymatic reduction.[39] The gold nanoparticles acted as photothermal agents that could generate heat under NIR irradiation, thereby triggering the release of the TNF-α payload. Notably, it was shown that the bacteria could accumulate in tumor tissue after oral administration.

Successful gene delivery for tumor therapy requires vectors capable of tumor-targeting as well as protection of the genetic material during transport.[40] Bacteria inherently have characteristics required for this purpose, including their ability to colonize tumors, self-propulsion, and environment-sensing abilities.[41] The term 'bactofection' refers to the use of a bacteria-based system for the transfer of plasmid DNA to mammalian cells after internalization.[27] Such a strategy allows for gene expression by a mammalian system, leading to improved protein translation compared to the direct use of bacterial cells.[41] In one case, *Salmonella choleraesuis* was used as a vector to specifically deliver the gene for thrombospondin-1, which promotes antiangiogenetic activity, to murine melanoma cells.[42] Following systemic administration, the bacteria accumulated preferentially in tumor tissue over the liver or spleen, which drove effective gene transfer and a high level of transgene expression. This ultimately led to delayed tumor growth and prolonged survival of the mice. Another study reported the use of *S. choleraesuis* as a vector to deliver a plasmid encoding the endostatin gene, also for antiangiogenic activity.[43] Following systemic administration, it was shown that attenuated *S. choleraesuis* bearing the endostatin expression plasmid colonized in the tumor area, reduced tumor growth, and extended survival by reducing both the intratumoral microvessel density and vascular endothelial growth factor expression.

Bactofection has also been utilized for RNA interference (RNAi)-based cancer therapy. RNAi is a gene silencing process wherein double-stranded RNA molecules delivered into the cytosolic compartment facilitate the degradation of target mRNAs.[44] In a study, nonpathogenic *E. coli* was genetically engineered to transcribe short hairpin RNA (shRNA).[45] Additionally, the plasmid encoded for protein factors enabled transfer of the shRNA into mammalian cells. Upon systemic administration, it was shown that platform was capable of silencing a specific cancer gene in the intestinal epithelium and tumor xenografts. Another payload that is often used for RNAi is small interfering RNA (siRNA), which possesses exceptional target specificity, but exerts only a transient effect on gene expression in proliferating cancer cells.[46] As a means to deliver antitumor siRNA in a sustained and localized manner, bacteria can be employed. *In vivo* studies demonstrated that siRNA could be effectively delivered to tumors following systemic intravenous administration of attenuated *Salmonella* Typhimurium engineered for this purpose.[47]

2.1.2 Magnetotaxis—Magnetotactic bacteria are motile gram-negative bacteria that have the ability to biomineralize magnetosomes, allowing the bacteria to orient along a magnetic field and move along an oxygen gradient.[48] One advantage of magnetically guided systems is that they can be controlled by long-range magnetic fields remotely and noninvasively.[27] Inspired by such properties, researchers have shown the potential use of magnetotactic bacteria for drug delivery. *Magnetospirillum marinus* MC-1, a flagellated magnetotactic bacterium, produces forces of ~4 pN that allows movement at speeds up to 100 body lengths per second, roughly 8 times the speed of a wild-type *E. coli* bacterium.

[49, 50] MC-1 bacteria have been exploited as a means to enhance the deposition of therapeutic agents into the tumor hypoxic region.[51] It was observed that the number of magnetically guided MC-1 in tumor sections following a peritumoral injection was much greater than that of bacteria without guidance. Furthermore, MC-1 bacteria bearing nanoliposomes also achieved deeper penetration into tumors under magnetic guidance and preferential accumulation in the hypoxic and necrotic areas, which showed the potential of this system to specifically deliver a variety of therapeutic compounds or imaging agents.

Along with penetrating tumor tissue, enhanced delivery to infectious biofilms can be mediated by magnetotactic bacteria. Biofilms are composed of a heterogenous group of bacterial colonies within a complex polymeric matrix. They can protect bacteria from a hostile environment, antibiotics, and the host's immune system, and their presence is often a cause of increased resistance of bacteria to treatment.[52] To enhance antibiotic delivery to infectious biofilms, drug-loaded microtubes were integrated into *Magnetospirillum gryphiswaldense* MSR-1 as a proof of concept.[53] For this biohybrid system, the release of the antibiotics was triggered by the acidic microenvironment of the biofilm, which could be penetrated by the propulsion ability of the MSR-1. While the efficacy of the proposed approach still requires additional validation *in vivo*, this study showed the potential of bacteria-powered biohybrids for addressing biofilms.

2.1.3 Bioreactors—Rather than simply considering bacteria as drug carriers, they can also be used as bioreactors that are capable of generating enzymes to trigger the conversion of an inactive prodrug into its biologically active form *in situ*.[54] Such a strategy can be taken advantage of in order to reduce inherent drug toxicities at off-target sites. In a recent study, *E. coli* was used to selectively produce a photothermal agent in the hypoxic tumor area (Figure 3).[55] A perylene diimide derivative-based supramolecular complex (CCPDI), which was loaded into a nanoliposome and co-delivered with the *E. coli*, could be converted into radical anions by hydrogenase on the surface of the bacteria. As the *E. coli* could preferentially colonize within the tumor hypoxic region and the nanoliposomes were engineered to release CCPDI within the tumor, highly selective photothermal therapy was enabled. Mice treated with the biohybrid platform showed significantly higher temperature and tumor ablation after laser irradiation. As another strategy, bacteria can be genetically engineered to provide source material for cytotoxic agent production. In one study, *E. coli* MG1655 was designed to express respiratory chain enzyme II, leading to increased H_2O_2 generation at the tumor site.[56] Magnetic iron oxide nanoparticles linked with bacteria could then serve as catalysts for the conversion of H_2O_2 into toxic hydroxyl radicals, inducing tumor apoptosis.

In another example of a bacteria-based bioreactor system, it was shown that natural dietary components can be leveraged as prodrugs.[57] Employing host-ingested components may eliminate the need for additional administration of precursors, thereby increasing patient compliance. In the study, *E. coli* Nissle was modified to exhibit affinity towards heparan sulphate proteoglycan on colorectal cancer cells and secrete the enzyme myrosinase. Following intraperitoneal injection, the engineered commensal microbes were localized in the colorectal tumor area and capable of converting diet-supplied glucosinolates to sulphoraphane, a potent anticancer agent. *In vivo* efficacy studies revealed that treatment

with these engineered bacteria significantly reduced tumor occurrence in the colorectal region of mice.

2.2 Bacterial Derivatives

2.2.1 Membrane Derivatives—In addition to whole bacteria, their individual components have shown potential as delivery vehicles.[58] These structures retain bacterial properties and functions while limiting the risk associated with the administration of live engineered bacteria.[58] Among these, extracellular vesicles have garnered considerable attention. These nanosized membrane structures are naturally shed by most bacteria and can play an important role in intercellular signaling. As such, researchers believe that they may hold prospects as drug delivery vectors for biomedical applications.[59] An intriguing idea has been to exploit naturally packed extracellular vesicles for treating bacterial infections.[60] This is based on findings that certain bacteria are known to load antimicrobial content into their extracellular vesicles.[60–62] While these observations were first discounted as passive effects of membrane budding events, recent data suggest the packaging of cargoes into extracellular vesicles and their subsequent release occur through a series of coordinated events, indicating that these antimicrobial packages may be strategically released to fight off bacterial competitors.[60, 63, 64] These “predatory” extracellular vesicles effectively lyse and kill a broad range of pathogenic bacteria,[62, 65, 66] and they have even been shown to outperform gentamycin treatment due to their inherent ability to fuse and release cargo inside infected cells.[62] Thus, such natural bacterial derivatives may provide new medical tools for fighting persistent infections in times of antibiotic resistance.[59, 62] Along with the use of natural membrane vesicles, other targeting specificities may be applied through genetic engineering of the parent bacteria. In one such example, it was shown that fusion of a human epidermal growth factor receptor 2 (EGFR2) affibody to *E. coli* outer membrane allowed for the production of tumor-targeting membrane vesicles.[67] Anticancer efficacy was demonstrated when these extracellular vesicles were loaded with therapeutic siRNA, leading to gene silencing and tumor regression.[67]

In addition to pure membrane vesicles, membrane-coated nanoparticles have proven themselves to be particularly useful in the fight against infectious diseases.[58, 68–70] These biomimetic nanoparticles can adapt various functions of the parent cell when cloaked in bacterial membrane.[70, 71] One of these functions includes the ability to be perceived as a pathogen, allowing them to exploit the unique interplay between bacteria and host immune cells to stimulate their own phagocytotic engulfment through natural uptake mechanisms. In one such approach, camouflaging nanoparticles with *E. coli* membrane was used to actively drive the loading of therapeutic cargo into live neutrophils.[72] Phagocytic cells such as neutrophils possess intrinsic abilities to sense and home to infectious sites through chemotactic behaviors, which was exploited in this study to generate self-guided cell micromotors.[73] To facilitate their loading, the nanoparticles were coated with bacterial membrane, leading to a significant increase in phagocytic uptake compared to that of uncoated nanoparticles.[72] In addition, when subjected to chemoattractants secreted by *E. coli*, the micromotors were shown to retain chemotactic behavior and actively move along the signaling gradient.[72]

Bacterial membrane mimics can be developed for treating difficult intracellular infections.[74] Severe cases of *Staphylococcus aureus* are often linked to the bacterium's ability to invade and hide inside host phagocytic cells, which generates a need for intracellular delivery to effectively target and treat such infections.[75] Taking advantage of the fact that infected phagocytes interact differently with certain pathogens,[76] antibiotic-loaded nanoparticles were cloaked in membrane isolated from *S. aureus* extracellular vehicles to promote their targeted uptake (Figure 4).[74] Neither coating with liposomes nor with *E. coli* outer membrane vesicles (OMVs) was able to reproduce similar uptake effects, demonstrating the specificity of the interaction. Moreover, upon intravenous injection into mice infected with *S. aureus*, the nanoparticles accumulated in major organs of infection, which in turn significantly reduced the bacterial burden at these sites. As the membrane coating was changed to OMVs isolated from *E. coli*, the targeting specificity skewed towards macrophages infected with the same pathogen, thus indicating that membrane coating strategies may be easily adjusted to match the intracellular pathogen in order to increase phagocytic delivery and effectively treat different infectious diseases.

2.2.2 Exotoxins and Spores—Gaining access to intracellular compartments is of crucial importance for not only the treatment of infectious diseases but also other diseases such as cancer, for which many therapeutic targets reside inside the cell.[77] Exotoxins are proteins that can be secreted by live bacteria or released upon bacterial death and lysis. These effector molecules have evolved to specifically bind and enter host cells, allowing them to circumvent endosomal degradation and inflict damage on intracellular targets.[78] This is often enabled by a modular composition, for which a binding moiety mediates cell receptor targeting and translocation of the enzymatically active subunit into the cell. Taking advantage of such highly effective translocation machineries, disarmed toxin conjugates have been used as transport vectors for cytosolic delivery of diverse therapeutic cargoes.[78] For instance, engineered anthrax fusion conjugates have been shown to effectively transport antibody mimics, oligonucleotides, and effector peptides or proteins into cells.[79–81] In addition, the enzymatic moieties of these toxins can themselves serve as potent drugs, which can be redirected to specific tissues by replacing the native binding domain with nonnative receptor targeting ligands.[82] In one translational example, the fusion of the human cytokine interleukin-2 to a diphtheria toxin fragment showed utility for anticancer therapy, and this construct has been granted Food and Drug Administration (FDA) approval for treatment of T cell lymphoma malignancies.[83] Similarly, a *Pseudomonas* exotoxin A-based immunotoxin fused to an anti-CD22 antibody has demonstrated clinical applications.[84]

Along with exotoxins, bacterial spores have gained much attention as vehicles in cancer therapy. Bacterial spores are seed-like structures carrying components of the parent cell embedded in a thick protein coat, which allows them to stay dormant until favorable environments present themselves and they can transform into live bacterial cells. Intriguingly, such physiological characteristics hold interesting opportunities for targeted drug delivery applications. In one instance, obligate anaerobe *Clostridium* spores were systemically administered to attain localized gene therapy within hypoxic tumors. In this approach, the *Clostridium* strain was genetically engineered to express prodrug-processing enzymes capable of converting nontoxic precursors into active drugs at the tumor site.[85, 86]

More specifically, spores baring a nitroreductase enzyme isolated from *Neisseria meningitidis* were able to process the prodrug CB1954 into DNA-alkylating *N*-hydroxylamine, leading to antitumor effects in a xenograft colon carcinoma model.[86]

In a slightly different approach, the physiological properties of a bacterial spore were used to generate *in situ* self-assembly nanoparticle manufacturing units for oral treatment of colorectal cancer.[87] The thick hydrophobic protein coat of probiotic *Bacillus coagulans* spores makes them resistant to harsh conditions and allows them to stay intact as they cross the low pH of the stomach. With these attributes in mind, it was suggested that the addition of a hydrophilic layer onto the coat would generate an amphiphilic shell that would drive nanoparticle self-assembly as the spore germinated and the coat disassembled in the intestines. To test this hypothesis, the spore surface was coated with deoxycholic acid to mediate endothelial transport, and chemotherapeutic drugs were attached through electrostatic interactions. As anticipated, cargo-loaded nanoparticles were shown to form in the intestines following oral administration of the modified spores. These nanoparticles showed enhanced intestinal adsorption compared to the drugs administered alone and significantly improved antitumor effects *in vivo*.

Another strategy for harnessing anaerobic bacterial spores is their use as guides for the delivery of therapeutic agents. Such a strategy contrasts with approaches that directly incorporate or attach payloads to bacteria. *Clostridium difficile* CCUG 37780, an anaerobic bacterial strain, is known to exist as spores under aerobic conditions and germinate in an anaerobic environment.[88] In a study, nanoparticles were decorated with anti-*Clostridium* polyclonal antibodies to target germinating *C. difficile* spores.[89] Following administration of the spores, subsequently injected antibody-conjugated nanoparticles were shown to accumulate in the hypoxic region of tumors. This approach outperformed a control, in which the payloads were attached directly to vegetative bacteria. To validate the *in vivo* therapeutic efficacy, upconversion nanorods and gold nanorods were delivered for bioimaging and photothermal therapy, respectively. Nanoparticles delivered by the antibody-directed method showed superior bioimaging and an enhanced photothermal therapeutic effect.

2.3 Bacteria-Mimicking Synthetics

The inherent abilities of microbes to target a host and navigate complex environments represent intriguing engineering opportunities and have inspired the development of synthetic bacteria-mimicking technologies for nanomedicine applications.[90] One common approach has been to adapt the shape and surface of nanocarriers to mimic that of pathogenic bacteria, thus exploiting certain bacteria–host interactions.[91–93] As an example, many respiratory pathogens use surface coatings rich in mannose to mediate their entry *via* surface receptors on alveolar macrophages.[94–96] Inspired by this principle, mannosylated iron-based metal–organic frameworks were explored for generating spherical and rod-like particles mimicking the overall properties of such airborne bacteria.[92] In *in vitro* tests, these showed efficient uptake into porcine alveolar macrophages and were further found to colocalize with intracellular mycobacteria at acidic cellular compartments. Synthetic bacteria-mimicking nanomaterials such as these, with their high drug loading and biological

targeting properties, may supplement natural derivatives in providing strategies for effective disease treatment.

Another field that has drawn great inspiration from microbial behavior is microrobots. Extensive work has explored the possibility of mimicking bacterial flagella-like propulsion to allow microrobots to move and overcome the viscous drag experienced by small-sized particles in low Reynolds number liquids.[97] One such approach is create magnetic helical microrobots with a size and shape similar to that of natural flagella.[97] These can be actuated to swim through corkscrew like motions under low magnetic fields and have established drug delivery[98] and *in vivo* tunability[99] potential, making them attractive tools for drug delivery applications. Recent efforts in this field have further focused on improving maneuverability. For example, the addition of multiple flagella components have allowed microrobots to exhibit run-and-tumble motions similar to those of multi-flagella microbes.[100] Other strategies have exploited hydrogel layering for generating soft micropropelling structures capable of adapting their morphologies to accommodate the alternating space and viscosities of biological systems.[101] In addition to their use as drug delivery vehicles alone, a recent study suggested that the convective flow generated by the propulsion of these micropropellers may be exploited to enhance nanoparticle transport at specific tissues.[102] The low diffusive transport of nanoparticles into tumors may greatly benefit from a convective fluid flow actively pulling the nanoparticles through endothelial gaps and into adjacent cancerous tissue. Exploring the feasibility of such a system, helical microswimmers were placed in a closed microfluidic system and actuated under a weak magnetic field. Under these conditions, the microswimmers generated a localized fluid flow that in turn was shown to significantly drive the penetration and accumulation of surrounding nanoparticles into collagen-rich pores (Figure 5).[102] Hence, the use of such wirelessly controlled micropropelling systems presents some attractive properties that may help address the clinical challenges of low nanoparticle accumulation in tumors.

3. IMMUNE MODULATION

The body's immune response to a bacterial infection is vast and complex. During the initial stages of infection, a host of immune cells are recruited to the site of infection, initiating a cascade of local inflammation.[103] Toll-like receptors (TLRs) and nucleotide-binding and oligomerization domain-like receptors on phagocytes identify microbial PAMPs present in and on bacteria, including biomolecules such as LPS, CpG DNA, and flagellin.[17, 104] Once these signaling pathways are triggered, innate immune cells initiate proinflammatory cytokine secretion and bacteria phagocytosis. Bacterial components are then processed and presented *via* major histocompatibility complexes (MHCs) to adaptive immune cells for generation of both cellular and humoral immunity against the identified antigens. It has become apparent to researchers that bacteria and their derivatives can effectively stimulate the immune system, and their utility for vaccine design has been heavily investigated. Similar to the principles of successful payload delivery, effective immunotherapy stems from successful trafficking to the immune cells of interest, controllable immune manipulation, and formulation safety without loss of function.[105–108]

3.1 Cancer Immunotherapy

Cancer immunotherapy has gained much interest in recent years as encouraging clinical data have emerged proving efficacy against a variety of malignancies.[109] Most prominently, successful strategies have focused on potentiating T cell antitumor responses through checkpoint inhibitors and adoptive chimeric antigen receptor T cell therapy.[110] Checkpoint inhibitors that have proven effective include those targeting programmed death-1/programmed death ligand-1 (PD-1/PD-L1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) molecules.[111] Tumors are known to take advantage of these immunosuppressive proteins for maintaining immune tolerance and their blocking by anti-PD1/PD-L1 and anti-CTLA-4 antibodies has led the FDA to approve such therapies for various tumor types.[111] Despite the undeniable clinical success of these immunotherapeutic strategies, many patients fail to benefit from them.[111] Poor efficacy is oftentimes correlated with low tumor antigenicity and a lack of immune cell infiltration, leaving such tumors insensitive to most cancer immunotherapies.[112]

Whereas tumors very closely resemble normal tissue and are lowly immunogenic, the immune system is highly trained to recognize and fight foreign microbes.[103] Accordingly, several studies have demonstrated the ability of bacteria and their derivatives to break immune tolerance and facilitate the recognition of tumor antigens.[2, 113–115] For example, intratumoral injections of attenuated bacteria have shown potential for turning immunologically “cold” tumors “hot” in a number of ways. These include immunomodulatory effects exerted on the tumor microenvironment, causing a depletion of immunosuppressive cells, including tumor-associated macrophages, myeloid-derived suppressors, and regulatory T cells, while increasing pro-immunostimulatory lymphocyte and $CD8^+$ T cell infiltrates within the tumor.[2] The inflammatory response further primes responses against tumor antigens by enhancing the display of costimulatory molecules essential for boosting T cell activation.[116, 117] Overall, bacteria and their derivatives are capable of stimulating multiple proinflammatory pathways,[118, 119] and they hold significant promise for potentiating antitumor responses against a wide range of cancer types.

3.1.1 Immune Stimulation—Most simply, live attenuated bacteria may act as a powerful immune stimulant to awaken both the innate and adaptive immune systems to an otherwise unseen tumor. A phase I clinical trial using attenuated *Salmonella* Typhimurium demonstrated its safety; however, the treatment unfortunately failed to elicit significant antitumor efficacy.[120] Other live bacteria are being investigated, including *Clostridium novyi*, and direct intratumoral injection of its spores was shown to elicit strong inflammatory responses and promote tumor regression.[121] Due to their obligate anerobic growth conditions, *C. novyi* spores did not dissipate out of the tumor and therefore reduced the risk of systemic bacterial infection. Successful preclinical studies in canines demonstrated a 37.5% objective response rate, which supported further study in a phase I clinical trial that established an acceptable safety profile for the *C. novyi* spores. This early success has led to another ongoing trial combining the treatment with an anti-PD1 checkpoint blockade in hopes of activating the immune system from multiple angles.[3] A variety of clinical trials with other immunostimulatory attenuated bacteria strains, including *Bifidobacterium longum*,[122] are currently ongoing.

Although bacteria themselves often elicit a strong immunostimulatory reaction, they can be further engineered as a gene delivery vehicle for cancer immunotherapy. Bactofection is a powerful strategy to introduce immunostimulatory genes into cancer cells where bacterial vectors are used to deliver a mammalian expression-controlled plasmid of interest into target cells.[123] This strategy allows for immune upregulation through cancer cell cytokine production, presentation of stimulatory markers, or immunoreactive antigen display.[40] Although many bacteria and target antigens have been explored,[124] efficiency remains a challenge in bactofection, as plasmid trafficking to the nucleus after bacterial engulfment is difficult to achieve. Bacteria-mediated delivery of mRNA may mitigate this concern, as mRNA is functional in the cell cytoplasm.[27]

While current clinical translation efforts have focused on whole attenuated bacteria, many research efforts are aimed at using extracellular vesicles as immune stimulants. OMVs produced from Gram-negative bacteria are strong candidates for *in situ* vaccine adjuvants, as their nanoscale size augments their transport and enhances their adjuvanticity. At the same time, they do not possess the same growth and infectious nature as their parent bacteria.[125] In one instance, *Salmonella* OMVs were used as an innate immunostimulatory agent and coated onto a prodrug-containing nanomicelle for combined antitumor immunotherapy and cytotoxic therapy.[126] When conjugated with a tumor-targeting peptide, the formulation accumulated heavily in tumor tissue, eliciting a robust antitumor immune response.

3.1.2 Antigen Delivery and Display—Often, adaptive immune cells are present but inactive at the tumor site. Vector-based antigen display and delivery is a promising strategy to enhance the immune response against an immunoevasive tumor.[127] Bacterial vectors are particularly attractive for this application because they are inherently adjuvanting and can also be employed to enhance antigen uptake and processing.[124] Along these lines, bacteria-inspired bottom-up strategies for antigen display have shown some potential. In one instance, bacterial components monophosphoryl lipid A, mifamurtide, flagellin, and CpG, all of which can stimulate various inflammatory pathways in innate immune cells, were synthesized and integrated into a liposomal nanoparticle.[128] When loaded with a model antigen ovalbumin (OVA), this bacteria-mimicking vector enhanced innate and adaptive immune responses in a B16-OVA tumor model and demonstrated a better safety profile than Freund's adjuvant, a gold standard in the field.

For a more top-down approach, tumor-associated antigen sequences can be readily integrated into the bacterial genetic code, allowing for expression and/or secretion of these heterologous proteins by the bacteria at the site of interest.[129, 130] Once injected into the body, the live bacteria can facilitate antigen presentation by mechanisms such as delivery *via* a type III secretion pathway or promoting MHC presentation after uptake by host cells.[124] *Listeria* has been particularly successful as a genetically engineered cancer vaccine vector and is currently being investigated in clinical trials for numerous cancers.[131]

Bacteria may also act as a vector for *ex vivo* vaccination strategies, such as those based on dendritic cells (DCs). Rod-shaped bacteria demonstrate the highest internalization rate by DCs when compared to spherical, chain, or 'Y' shaped bacteria.[132] *Lactobacillus*, a rod-shaped bacterium, can be curated into a vaccine vector by hollowing it out through

hydrothermal treatment, allowing for insertion of tumor-associated antigens. This biomimetic platform maintains the original bacterium's native binding and uptake characteristics while also eliciting strong antitumor immunity against the inserted antigen.[132] Similarly, bacterial ghosts have been produced by insertion of lysis protein E in bacteria, allowing for controllable production and collection of bacterial shells which do not contain cytoplasmic content but preserve all the surface properties of the host. As a result, bacterial ghosts provide a safer bacterial vector while maintaining their immunostimulatory properties, making them suitable for vaccine applications.[133] These bacterial ghosts can be passively loaded with entire oncolysates, removing the need to identify specific tumor antigens and increasing antigenic diversity.[134, 135]

Bacterial OMV vectors offer flexible delivery, safety due to their inability to replicate, and enhanced uptake and processing in APCs due to their nanoscale size.[136] OMV-based anticancer vaccines using materials from different bacterial origins have been validated in a wide range of cancer models.[125, 136, 137] As an example, *Salmonella* OMVs have been fused with cancer cell membrane vesicles, thereby integrating tumor antigens and immunostimulatory compounds into a single nanoplatform that could be used to successfully vaccinate mice.[138] Combining additional modalities such as radiation therapy with these extracellular vesicle-based platforms may further enhance antitumor efficacy. In one example, maleimide-functionalized bacterial OMVs were coated onto nanoparticle cores containing PC7A and CpG, and the resulting formulation was administered to solid tumors post-radiation therapy (Figure 6).[139] The radiation therapy generated a host of neoantigens that could be directly adsorbed onto the nanoparticle *via* the maleimide surface groups. Once adsorbed, these neoantigens were more likely to be taken up by DCs, aided by the immunogenic nature of the OMVs. After uptake, the PC7A in the core of the nanoparticle facilitated endosomal disruption and CpG, a TLR9 agonist, acted as an additional adjuvant to promote immune processing and presentation of the tumor neoantigens. Ultimately, this bacteria-mimetic vector was able to successfully enhance immune activation and produced an antitumor memory response.

3.1.3 Nanobody Delivery—Nanobodies have become a promising tool for immunotherapy, as the single domain structures are easier to produce than traditional antibodies and also exhibit relatively low immunogenicity.[140] While their small size allows for increased tumor penetration and enhanced activity, nanobodies also suffer from short serum half-lives.[140] Bacteria-based delivery vehicles have been employed to overcome some of the challenges facing the use of nanobodies alone. In one example, quorum-sensing bacteria, equipped with a lysing mechanism to control growth, were used to deliver checkpoint blockade nanobodies for anticancer treatment (Figure 7).[141] The bacteria were able to address the short half-life of nanobodies by continuously producing them exclusively at the site of the tumor, thereby enhancing the local concentration of the therapy without generating systemic toxicity. The ability of the treatment to generate systemic antitumor responses was evaluated in a bilateral tumor model, and it was demonstrated that adaptive immunity generated against tumor antigens in the treated flank resulted in control of cancer growth elsewhere in the body.

In addition to checkpoint blockades, nanobodies have also been directed against markers such as CD47, which is a 'don't eat me' self-marker that reduces phagocytosis.[142] Although useful to protect against autoimmunity, CD47 is commonly overexpressed on cancer cell surfaces.[143] Treatment with anti-CD47 may therefore increase phagocytosis of cancer cells, enhancing immune reactivity and promoting cross-presentation of tumor antigens. However, anti-CD47 antibodies suffer from toxicity limitations, leading to the phagocytosis of erythrocytes and platelets, and they also require high saturation levels in tumor tissue for efficacy.[144] Recently, it was shown that delivery of anti-CD47 nanobodies using bacteria could alleviate these issues by localizing therapy to the tumor site.[145] Tumor regression was achieved with the use of lysis circuit-controlled bacteria producing a CD47 nanobody when administered both intratumorally and intravenously, with the latter demonstrating limited systemic toxicity due to tumor targeting and controlled nanobody production.

3.2 Antibacterial Immune Modulation

3.2.1 Clinical Status—Bacterial vaccines account for more than one third of vaccines approved by the United States FDA, second only to viral vaccines.[146] They can be divided roughly into three types: live attenuated vaccines, killed or inactivated vaccines, and subunit vaccines. Historically, live bacteria have been used as vaccines since 1884, when attenuated *Vibrio cholera* was injected subcutaneously in order to obtain immunity against the bacteria.[147] It is possible for live attenuated vaccines to generate lifelong protection with only one or two doses, but they may also cause sickness in people with weakened immune systems and require refrigeration.[148] Examples of live attenuated vaccines are the BCG vaccine and the typhoid fever vaccine, Vivotif.[149] Inactivated vaccines are safer and can be easily stored in lyophilized form, but they often require multiple doses to achieve the desired level of protection.[150] One example of an inactivated vaccine is the whole cell pertussis vaccine using killed *Bordetella pertussis*.[151] Subunit vaccines generally have an improved safety profile compared to inactivated bacteria and account for most clinically available bacterial vaccines. They can be further divided into toxoid vaccines, including those for diphtheria and tetanus, and conjugate vaccines such as PedvaxHIB against the *Haemophilus influenzae* type B bacteria.[152] Subunit vaccines oftentimes require booster doses and are difficult to produce for many pathogens. While vaccines play an important role in the clinical management of infectious diseases, there is no effective vaccine for many of the most dangerous bacterial infections. As such, many researchers have begun to utilize various biomaterials to enhance vaccine design,[153] and those that are bacteria-derived or bacteria-inspired have played a prominent role.

3.2.2 Engineered Live Bacteria—Various strategies have been studied to enable the development of more effective live attenuated bacterial vaccines. One example is the use of multiple *Pseudomonas aeruginosa* strains to design a vaccine that prevented against acute lung infections caused by the pathogen.[154] A total of 19 attenuated strains were tested, and a combination of 3 to 4 of strains that had different LPS serogroups resulted in the best protection. Due to the multivalency of this approach, protection against a broad spectrum of *P. aeruginosa* strains could be achieved with a single formulation. Another strategy involved the use of engineered *Mycobacterium tuberculosis* as a vaccine against tuberculosis.[155] Deletion of the SigH gene, which is responsible for inducing antioxidant production to

protect against oxidative stress, abrogated pathogenicity. This led to a safer bacterial strain that was still able to promote formation of inducible bronchus-associated lymphoid tissue, as well as recruitment of $CD4^+$ and $CD8^+$ T cells to the lungs. A more generally applicable approach for attenuating pathogenic bacteria is by engineering D-glutamate auxotrophs.[156] Since D-glutamate is crucial for forming the bacterial cell wall, the auxotrophs exhibit attenuated virulence and limited growth. The effectiveness and versatility of this type of approach has been demonstrated in different pathogens, including *Acinetobacter baumannii*, *P. aeruginosa*, and *S. aureus*. Overall, using live bacteria for developing vaccines leads to potent and inherently multiantigenic formulations where identification of individual antigens is not required.

3.2.3 Bacterial Ghosts—Compared with live bacteria, a safer alternative for vaccine development is the use of bacterial ghosts, which consist of empty cell envelopes of Gram-negative bacteria.[157, 158] One approach for generating bacteria ghosts is through the controlled expression of lysis gene *E* of the bacteriophage ΦX174, which has been used to create a transmembrane tunnel structure on the surface of *E. coli* to drain away the intracellular contents. Although initial developments were focused mostly on *E. coli*, it has been demonstrated that gene *E* was able to induce lysis and generate ghosts using other Gram-negative bacteria as well. Just like live bacteria vaccines, bacterial ghosts can not only directly activate immune cells such as DCs, macrophages, and B or T cells, but they can also activate epithelial cells, fibroblasts, and keratinocytes, all of which can then attract immune cells.[159] The biggest advantage of using ghosts over live bacteria vaccines is their improved safety profile, as there is no danger of them reverting back to a pathogenic form. Also, since ghosts can preserve the epitopes characteristic of virulent bacteria strains, they may be able to induce higher quality immune responses compared to weakened or fragmented bacteria.

Bacterial ghosts have been used in various animal models to prove their potential as vaccines against bacterial infections. For example, a vaccine using the ghosts of *Bordetella bronchiseptica*, a Gram-negative bacterium that can infect canines as well as immunocompromised humans, was proven to be effective against respiratory infections in dogs.[160] In the study, a stable and ready-to-use liquid formulation of the bacterial ghost vaccine was developed. The liquid formulation had its advantages in that it aligned well with veterinary practices of storing vaccines in liquid form rather than freeze-dried. Even at a lower dosage compared to the licensed vaccine Bronchicine CAe, subcutaneous injection of the ghosts resulted in similar efficacy and safety. In another study, ghosts from *E. coli* were used to stimulate the immune systems of mice against *M. tuberculosis*.[161] The immunostimulatory effect of the ghosts was shown to be far greater than that of LPS. It is believed that this was due to the fact that, by replicating more features of bacteria, the ghosts were able to engage the immune system in a variety of ways. In addition, the ability of the ghosts to synergize with other treatments was explored by co-administering with commercially available anti-tuberculosis drugs such as bedaquiline and delamanid. It was demonstrated that bactericidal efficacy was increased compared to administration of the drugs alone.

3.2.4 Outer Membrane Vesicles—Bacteria can produce and spontaneously release OMVs, which contain cargoes including toxins and enzymes, to interact with the surrounding environment.[162] Their nanosized structure, as well as the presence of bacterial antigens and various PAMPs, make OMVs intriguing vaccine candidates.[8, 163] Serogroup B meningococcal disease has been targeted by OMV vaccines, and the clinically used BEXSERO formulation contains recombinant bacterial antigens combined with OMVs to elicit strong immune responses.[164] In the production of BEXSERO, the detergent deoxycholate is used to kill bacteria and mediate vesicle formation.[165] This approach can weaken immunogenicity while causing aggregation and contamination,[166] and thus it may be beneficial for next generation OMV vaccines to employ native spontaneously released OMVs to avoid the use of detergent.[167]

Due to their high immunogenicity, OMVs can be engineered as vaccine platforms that, when fused with weakly immunogenic heterologous antigens, can elicit potent and specific immune responses. Several genetic fusion systems have been explored to express foreign antigens on the surface of OMVs. In one example, *E. coli* bacteria were engineered such that the cytolysin A protein found on their OMVs was fused with a poorly immunogenic green fluorescent protein (GFP).[168] It was then demonstrated that vaccination with OMVs derived from the modified bacteria could strongly elicit GFP-specific antibodies. Using the same approach, the outer membrane protein Omp22 of *A. baumannii* has been fused to ClyA on *E. coli* OMVs, and the resulting formulation was able to protect mice against *A. baumannii* infection.[169] A fusion system based on factor H binding protein, a meningococcal surface lipoprotein, was developed specifically to express heterologous lipoproteins on the surface of OMVs.[170] To validate this system, borrelial outer surface protein A was successfully engineered onto the surface of meningococcal OMVs, and vaccination with the formulation elicited antibody titers against the displayed protein. In addition to surface expression, heterologous antigens can be carried within the lumen of OMVs. In one case, recombinant *E. coli* OMVs were designed with streptolysin O fused to the luminal side of outer membrane protein A and were shown to protect mice against challenge with Group A *Streptococcus*.[171]

Various nanoplatforms have been developed to modulate the delivery of OMV-based vaccines. For mucosal vaccination, when encapsulated into nanoparticles, OMVs can be protected from extreme environmental conditions and more easily be captured by mucosal APCs to improve immune responses. For instance, after loading into nanoparticles made from a copolymer of methyl vinyl ether and maleic anhydride, *Shigella flexneri* OMVs administered by the nasal or oral route could provide long-term protection and improve the survival rate of mice challenged with the bacteria.[172] The bioadhesion of the copolymer and the prolonged release of the antigens after encapsulation may explain the benefits of this system. After encapsulation into zein nanoparticles with a hydrophilic mannosamine-poly(anhydride) corona, OMVs derived from enterotoxigenic *E. coli* could be administered orally and elicited stronger innate and humoral immune responses in mice and pregnant sows compared to free OMVs.[173]

In contrast to platforms for nanoencapsulation, OMV-coated nanoparticles are an emerging technology that leverages the tunable physicochemical properties of nanomaterials while

preserving the surface display of native bacterial antigens.[174] As a proof of concept, *E. coli* OMVs were coated onto the surface of gold nanoparticles and used to modulate antibacterial immunity (Figure 8).[71] The resulting nanoformulation efficiently drained to the lymph nodes, activating the resident immune cells. Compared with OMVs only, the OMV-coated gold nanoparticles could elicit stronger antibody responses with higher avidity. In another membrane coating example, OMVs were coated onto crosslinked bovine serum albumin nanoparticles, and the formulation was shown to enhance protection against fatal infection by carbapenem-resistant *Klebsiella pneumoniae*.[175] Overall, OMVs have demonstrated significant potential as antibacterial vaccine candidates, although scalable production and the balancing of immunogenicity with toxicity remain challenging.[176]

3.2.5 Nanotoxoids—For many bacterial infections such as diphtheria and tetanus, symptoms are driven by the production of secreted toxins. Inactivated versions of these toxins, also referred to as toxoids, have been used to vaccinate against some bacterial infections in the clinic.[177] Traditional methods for toxoid production include heat and chemical inactivation,[178] both of which may disrupt the structure of the original toxins and reduce vaccine efficacy.[179] In order to make toxoid vaccines less toxic while maintaining epitopic integrity, recombinantly modified toxoids as well as nanoparticle-based platforms have been developed.[180–182] For instance, chitosan–dextran sulfate nanoparticles were constructed to co-load the pertussis toxoid and the adjuvant immunoglobulin A for sustained release, and it was shown that the formulation could induce enhanced IgG responses in mice compared to the conventional toxoid formulation with alum as the adjuvant.[183]

Recently, a nanotoxoid platform has been developed where toxins are detained on cell membrane-coated nanoparticles, and the resulting complex is used as a vaccine.[184] The basis of nanotoxoids is the strong interactions between cell membranes and bacterial virulence factors such as pore-forming toxins and neurotoxins.[185] The detained toxins are inactivated while their native structure remains undenatured, enabling their use as safe and effective toxoid vaccines.[186] Nanotoxoids can be divided simply into single and multiple toxin systems. The first reported nanotoxoid formulation consisted of staphylococcal α-hemolysin loaded onto erythrocyte membrane-coated polymeric nanoparticles, and the formulation showed less toxicity and induced more toxin-specific antibody titers than a heat-inactivated control.[184] Further study revealed vaccination with the nanotoxoids could inhibit lesion formation and reduce bacterial burden in the major organs of mice challenged subcutaneously with methicillin-resistant *S. aureus* (MRSA).[187] Multiple toxin systems provide a more complete response against the plethora of toxins secreted by bacterial pathogens. A facile method to produce these multi-antigenic formulations is to incubate cell membrane-coated nanoparticles with secreted proteins collected from bacterial culture supernatant. In one study, hemolytic secreted proteins of MRSA, including α-hemolysin, γ-hemolysin, and Panton–Valentine leucocidin, were collected and incubated with red blood cell membrane-coated nanoparticles, yielding a nanotoxoid that was safer and elicited enhanced immune responses as compared to heat-inactivated proteins.[188] Another study explored the possibility of utilizing macrophage membrane coating to capture secreted proteins from multidrug-resistant Gram-negative *P. aeruginosa* (Figure 9).[189] Due to their role in the body's defense against bacteria, macrophages bind a number of antigens secreted

by *P. aeruginosa*, and this was leveraged by the macrophage membrane-coated nanoparticles to capture antigens such as flagellin and various outer membrane proteins. After intranasal administration, the nanotoxoid was able to elicit potent immune responses against *P. aeruginosa* and protected mice against live bacterial infection.

To enhance vaccine efficacy upon oral administration, micromotor technology has been leveraged together with cell membrane coating to fabricate toxoids with active propulsion characteristics. In an example, a biomimetic micromotor was made by coating red blood cell membrane, with α-hemolysin preinserted into it, onto a magnesium-based Janus core.[190] The formulation was further protected with an enteric coating, which allowed it to safely travel through the harsh acidic environment of the stomach, after which the propulsion would activate and drive the vaccine payload toward the mucosal lining of the intestines. The motorized toxoids elicited improved mucosal immune responses as compared to static microparticles when administered orally. Overall, nanotoxoid technology has shown great potential for antibacterial vaccine applications, and it can be easily generalized to any type of pathogen that secretes cell-attacking virulence factors.[191] It should be noted that the cell membrane coatings can be sourced from a variety of cell types,[192–199] providing additional design flexibility.

4. CONCLUSION

Bacteria and bacteria-inspired nanomaterials have shown great potential for biomedical applications based on their unique properties. Live bacteria provide propulsion and bacterial taxis, both of which can be used to help direct a therapy to a site of interest and enhance penetration into solid tissues. In addition, bacteria are responsive to their environment, and they can be engineered for tunable growth and triggered payload release. The native immunogenicity of bacteria and their derivatives offers another valuable asset, enabling them to be leveraged as powerful adjuvants and smart vaccine vectors. This review has summarized the current field of bacteria-inspired nanomedicine, with a focus on payload delivery and immune modulation. The more traditional applications of bacteria, including *in situ* immune stimulation and tumor-tropic delivery, have given way to more elegant biohybrids with added functionalities and synthetic constructs that successfully mimic some of the unique properties of bacteria. Various bacteria-derived platforms, including those based on bacterial ghosts, OMVs, toxins, spores, and cell membrane-coated nanoparticles, can effectively interface with the body and utilize bacteria-specific properties for smart delivery and vaccination.

Nevertheless, there are challenges that remain, and these will necessitate further engineering and innovation to overcome. A unique challenge of bacteria-based therapeutics is dosage considerations; in the case of live bacteria, even when under quorum-sensing lysis control, the dosage administered and the scale of bacterial growth inside the body may not be consistent among patients. This variation may necessitate the use of fail-safes such as kill switches[200] and genetic firewalls[201, 202] that are capable of curbing bacterial proliferation, as well as a deeper understanding of growth kinetics. Due to their biological nature, the translation of bacteria-inspired therapeutics to the clinic will also require a robust framework to ensure functional stability throughout the production process. As many of the systems

discussed in this review are highly novel with no analogous products on the market, careful collaboration with regulatory agencies will be necessary to ensure proper manufacturing practices and to guarantee patient safety. Current clinical trials have only scratched the surface of bacteria-inspired medicine. There is still much to learn about bacteria and how they interact with the human body, and our ever-growing knowledge on these topics will help to inform the design of future nanomedicine platforms that could significantly change how infectious diseases or cancers are managed in the clinic.

ACKNOWLEDGEMENTS

This work is supported by the Defense Threat Reduction Agency Joint Science and Technology Office for Chemical and Biological Defense under Grant Number HDTRA1-18-1-0014 and the National Institutes of Health under Award Number R01CA200574. J.H.P. is supported by a National Institutes of Health 5T32CA153915 training grant from the National Cancer Institute.

REFERENCES

1. Coley WB II. Contribution to the Knowledge of Sarcoma. Ann. Surg 1891, 14 (3), 199–220.
2. Kaimala S; Al-Sbiei A; Cabral-Marques O; Fernandez-Cabezudo MJ; Al-Ramadi BK, Attenuated Bacteria as Immunotherapeutic Tools for Cancer Treatment. Front. Oncol 2018, 8, 136. [PubMed: 29765907]
3. Zhou S; Gravekamp C; Bermudes D; Liu K, Tumour-Targeting Bacteria Engineered to Fight Cancer. Nat. Rev. Cancer 2018, 18 (12), 727–743. [PubMed: 30405213]
4. Kawai K; Miyazaki J; Joraku A; Nishiyama H; Akaza H, Bacillus Calmette-Guerin (BCG) Immunotherapy for Bladder Cancer: Current Understanding and Perspectives on Engineered BCG Vaccine. Cancer Sci 2013, 104 (1), 22–27. [PubMed: 23181987]
5. Riglar DT; Silver PA, Engineering Bacteria for Diagnostic and Therapeutic Applications. Nat. Rev. Microbiol 2018, 16 (4), 214–225. [PubMed: 29398705]
6. Duong MT; Qin Y; You SH; Min JJ, Bacteria-Cancer Interactions: Bacteria-Based Cancer Therapy. Exp. Mol. Med 2019, 51 (12), 1–15.
7. Lin IY; Van TT; Smooker PM, Live-Attenuated Bacterial Vectors: Tools for Vaccine and Therapeutic Agent Delivery. Vaccines 2015, 3 (4), 940–972. [PubMed: 26569321]
8. Kaparakis-Liaskos M; Ferrero RL, Immune Modulation by Bacterial Outer Membrane Vesicles. Nat. Rev. Immunol 2015, 15 (6), 375–387. [PubMed: 25976515]
9. Whiteley M; Diggle SP; Greenberg EP, Progress in and Promise of Bacterial Quorum Sensing Research. Nature 2017, 551 (7680), 313–320. [PubMed: 29144467]
10. Apostolico Jde S; Lunardelli VA; Coirada FC; Boscardin SB; Rosa DS, Adjuvants: Classification, Modus Operandi, and Licensing. J. Immunol. Res 2016, 2016, 1459394. [PubMed: 27274998]
11. St Jean AT; Swofford CA; Panteli JT; Brentzel ZJ; Forbes NS, Bacterial Delivery of *Staphylococcus aureus* α-Hemolysin Causes Regression and Necrosis in Murine Tumors. Mol. Ther 2014, 22 (7), 1266–1274. [PubMed: 24590046]
12. Chen J; Yang B; Cheng X; Qiao Y; Tang B; Chen G; Wei J; Liu X; Cheng W; Du P; Huang X; Jiang W; Hu Q; Hu Y; Li J; Hua ZC, *Salmonella*-Mediated Tumor-Targeting TRAIL Gene Therapy Significantly Suppresses Melanoma Growth in Mouse Model. Cancer Sci 2012, 103 (2), 325–333. [PubMed: 22054098]
13. Toley BJ; Forbes NS, Motility is Critical for Effective Distribution and Accumulation of Bacteria in Tumor Tissue. Integr. Biol 2012, 4 (2), 165–176.
14. Krell T; Lacal J; Munoz-Martinez F; Reyes-Darias JA; Cadirci BH; Garcia-Fontana C; Ramos JL, Diversity at Its Best: Bacterial Taxis. Environ. Microbiol 2011, 13 (5), 1115–1124. [PubMed: 21087385]
15. Kasinskas RW; Forbes NS, *Salmonella* Typhimurium Lacking Ribose Chemoreceptors Localize in Tumor Quiescence and Induce Apoptosis. Cancer Res. 2007, 67 (7), 3201–3209. [PubMed: 17409428]

16. Fernández-Medina M; Ramos-Docampo MA; Hovorka O; Salgueiriño V; Städler B, Recent Advances in Nano- and Micromotors. Adv. Funct. Mater 2020, 30 (12), 1908283.
17. Hajam IA; Dar PA; Shahnawaz I; Jaume JC; Lee JH, Bacterial Flagellin—A Potent Immunomodulatory Agent. Exp. Mol. Med 2017, 49 (9), e373. [PubMed: 28860663]
18. Skidmore BJ; Chiller JM; Morrison DC; Weigle WO, Immunologic Properties of Bacterial Lipopolysaccharide (LPS): Correlation between the Mitogenic, Adjuvant, and Immunogenic Activities. J. Immunol 1975, 114 (2 Pt 2), 770–775. [PubMed: 46249]
19. Olvera-Gomez I; Hamilton SE; Xiao ZG; Guimaraes CP; Ploegh HL; Hogquist KA; Wang LC; Jameson SC, Cholera Toxin Activates Nonconventional Adjuvant Pathways That Induce Protective CD8 T-cell Responses after Epicutaneous Vaccination. Proc. Natl. Acad. Sci. U. S. A 2012, 109 (6), 2072–2077. [PubMed: 22308317]
20. Bahrami B; Hojjat-Farsangi M; Mohammadi H; Anvari E; Ghalamfarsa G; Yousefi M; Jadidi-Niaragh F, Nanoparticles and Targeted Drug Delivery in Cancer Therapy. Immunol. Lett 2017, 190, 64–83. [PubMed: 28760499]
21. Dehaini D; Fang RH; Zhang L, Biomimetic Strategies for Targeted Nanoparticle Delivery. Bioeng. Transl. Med 2016, 1 (1), 30–46. [PubMed: 29313005]
22. Park JH; Dehaini D; Zhou J; Holay M; Fang RH; Zhang L, Biomimetic Nanoparticle Technology for Cardiovascular Disease Detection and Treatment. Nanoscale Horiz. 2020, 5 (1), 25–42. [PubMed: 32133150]
23. Zhuang J; Gong H; Zhou J; Zhang Q; Gao W; Fang RH; Zhang L, Targeted Gene Silencing *In Vivo* by Platelet Membrane-Coated Metal-Organic Framework Nanoparticles. Sci. Adv 2020, 6 (13), eaaz6108. [PubMed: 32258408]
24. Tran S; DeGiovanni PJ; Piel B; Rai P, Cancer Nanomedicine: A Review of Recent Success in Drug Delivery. Clin. Transl. Med 2017, 6 (1), 44. [PubMed: 29230567]
25. Gao W; Chen Y; Zhang Y; Zhang Q; Zhang L, Nanoparticle-Based Local Antimicrobial Drug Delivery. Adv. Drug Deliv. Rev 2018, 127, 46–57. [PubMed: 28939377]
26. Akin D; Sturgis J; Ragheb K; Sherman D; Burkholder K; Robinson JP; Bhunia AK; Mohammed S; Bashir R, Bacteria-Mediated Delivery of Nanoparticles and Cargo into Cells. Nat. Nanotechnol 2007, 2 (7), 441–449. [PubMed: 18654330]
27. Hosseinidoust Z; Mostaghaci B; Yasa O; Park BW; Singh AV; Sitti M, Bioengineered and Biohybrid Bacteria-Based Systems for Drug Delivery. Adv. Drug Deliv. Rev 2016, 106 (Pt A), 27–44. [PubMed: 27641944]
28. Wang CZ; Kazmierczak RA; Eisenstark A, Strains, Mechanism, and Perspective: *Salmonella*-Based Cancer Therapy. Int. J. Microbiol 2016, 2016, 5678702. [PubMed: 27190519]
29. Chen W; Wang Y; Qin M; Zhang X; Zhang Z; Sun X; Gu Z, Bacteria-Driven Hypoxia Targeting for Combined Biotherapy and Photothermal Therapy. ACS Nano 2018, 12 (6), 5995–6005. [PubMed: 29786420]
30. Wang C; Xu L; Liang C; Xiang J; Peng R; Liu Z, Immunological Responses Triggered by Photothermal Therapy with Carbon Nanotubes in Combination with Anti-CTLA-4 Therapy to Inhibit Cancer Metastasis. Adv. Mater 2014, 26 (48), 8154–8162. [PubMed: 25331930]
31. Chen Q; Xu L; Liang C; Wang C; Peng R; Liu Z, Photothermal Therapy with Immune-Adjuvant Nanoparticles Together with Checkpoint Blockade for Effective Cancer Immunotherapy. Nat. Commun 2016, 7, 13193. [PubMed: 27767031]
32. Agrawal N; Bettegowda C; Cheong I; Geschwind JF; Drake CG; Hipkiss EL; Tatsumi M; Dang LH; Diaz LA; Pomper M; Abusedera M; Wahl RL; Kinzler KW; Zhou S; Huso DL; Vogelstein B, Bacteriolytic Therapy Can Generate a Potent Immune Response against Experimental Tumors. Proc. Natl. Acad. Sci. U. S. A 2004, 101 (42), 15172–15177. [PubMed: 15471990]
33. Cheong I; Huang X; Bettegowda C; Diaz LA; Kinzler KW; Zhou S; Vogelstein B, A Bacterial Protein Enhances the Release and Efficacy of Liposomal Cancer Drugs. Science 2006, 314 (5803), 1308–1311. [PubMed: 17124324]
34. Chen F; Zang Z; Chen Z; Cui L; Chang Z; Ma A; Yin T; Liang R; Han Y; Wu Z; Zheng M; Liu C; Cai L, Nanophotosensitizer-Engineered *Salmonella* Bacteria with Hypoxia Targeting and Photothermal-Assisted Mutual Bioaccumulation for Solid Tumor Therapy. Biomaterials 2019, 214, 119226. [PubMed: 31174068]

35. Homayun B; Lin X; Choi HJ, Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. Pharmaceutics 2019, 11 (3), 129.
36. Morishita M; Peppas NA, Is the Oral Route Possible for Peptide and Protein Drug Delivery? Drug Discov. Today 2006, 11 (19–20), 905–910. [PubMed: 16997140]
37. Bernardes N; Chakrabarty AM; Fialho AM, Engineering of Bacterial Strains and Their Products for Cancer Therapy. Appl. Microbiol. Biotechnol 2013, 97 (12), 5189–5199. [PubMed: 23644748]
38. Zhou X; Zhang X; Han S; Dou Y; Liu M; Zhang L; Guo J; Shi Q; Gong G; Wang R; Hu J; Li X; Zhang J, Yeast Microcapsule-Mediated Targeted Delivery of Diverse Nanoparticles for Imaging and Therapy *via* the Oral Route. Nano. Lett 2017, 17 (2), 1056–1064. [PubMed: 28075596]
39. Fan JX; Li ZH; Liu XH; Zheng DW; Chen Y; Zhang XZ, Bacteria-Mediated Tumor Therapy Utilizing Photothermally-Controlled TNF-α Expression *via* Oral Administration. Nano. Lett 2018, 18 (4), 2373–2380. [PubMed: 29558152]
40. Baban CK; Cronin M; O'Hanlon D; O'Sullivan GC; Tangney M, Bacteria as Vectors for Gene Therapy of Cancer. Bioeng. Bugs 2010, 1 (6), 385–394. [PubMed: 21468205]
41. Forbes NS, Engineering the Perfect (Bacterial) Cancer Therapy. Nat. Rev. Cancer 2010, 10 (11), 785–794. [PubMed: 20944664]
42. Lee CH; Wu CL; Shiau AL, Systemic Administration of Attenuated *Salmonella choleraesuis* Carrying Thrombospondin-1 Gene Leads to Tumor-Specific Transgene Expression, Delayed Tumor Growth and Prolonged Survival in the Murine Melanoma Model. Cancer Gene Ther 2005, 12 (2), 175–184. [PubMed: 15375381]
43. Lee CH; Wu CL; Shiau AL, Endostatin Gene Therapy Delivered by *Salmonella choleraesuis* in Murine Tumor Models. J. Gene Med 2004, 6 (12), 1382–1393. [PubMed: 15468191]
44. Hannon GJ, RNA Interference. Nature 2002, 418 (6894), 244–251. [PubMed: 12110901]
45. Xiang S; Fruehauf J; Li CJ, Short Hairpin RNA-Expressing Bacteria Elicit RNA Interference in Mammals. Nat. Biotechnol 2006, 24 (6), 697–702. [PubMed: 16699500]
46. Tuschl T; Borkhardt A, Small Interfering RNAs: A Revolutionary Tool for the Analysis of Gene Function and Gene Therapy. Mol. Interv 2002, 2 (3), 158–167. [PubMed: 14993376]
47. Zhang L; Gao L; Zhao L; Guo B; Ji K; Tian Y; Wang J; Yu H; Hu J; Kalvakolanu DV; Kopecko DJ; Zhao X; Xu DQ, Intratumoral Delivery and Suppression of Prostate Tumor Growth by Attenuated *Salmonella enterica* Serovar Typhimurium Carrying Plasmid-Based Small Interfering RNAs. Cancer Res. 2007, 67 (12), 5859–5864. [PubMed: 17575154]
48. Bazylinski DA; Williams TJ; Lefèvre CT; Berg RJ; Zhang CL; Bowser SS; Dean AJ; Beveridge TJ, *Magnetococcus marinus* Gen. Nov., Sp. Nov., a Marine, Magnetotactic Bacterium That Represents a Novel Lineage (*Magnetococcaceae* Fam. Nov., *Magnetococcales* Ord. Nov.) at the Base of the *Alphaproteobacteria*. Int. J. Syst. Evol. Microbiol 2013, 63 (Pt 3), 801–808. [PubMed: 22581902]
49. Carlsen RW; Sitti M, Bio-Hybrid Cell-Based Actuators for Microsystems. Small 2014, 10 (19), 3831–3851. [PubMed: 24895215]
50. Andersson M; Fällman E; Uhlin BE; Axner O, Dynamic Force Spectroscopy of *E. coli* P Pili. Biophys. J 2006, 91 (7), 2717–2725. [PubMed: 16844748]
51. Felfoul O; Mohammadi M; Taherkhani S; de Lanauze D; Zhong Xu Y; Loghin D; Essa S; Jancik S; Houle D; Lafleur M; Gaboury L; Tabrizian M; Kaou N; Atkin M; Vuong T; Batist G; Beauchemin N; Radzioch D; Martel S, Magneto-Aerotactic Bacteria Deliver Drug-Containing Nanoliposomes to Tumour Hypoxic Regions. Nat. Nanotechnol 2016, 11 (11), 941–947. [PubMed: 27525475]
52. Sharma D; Misba L; Khan AU, Antibiotics versus Biofilm: An Emerging Battleground in Microbial Communities. Antimicrob. Resist. Infect. Control 2019, 8, 76. [PubMed: 31131107]
53. Stanton MM; Park BW; Vilela D; Bente K; Faivre D; Sitti M; Sánchez S, Magnetotactic Bacteria Powered Biohybrids Target *E. coli* Biofilms. ACS Nano 2017, 11 (10), 9968–9978. [PubMed: 28933815]
54. Lehouritis P; Springer C; Tangney M, Bacterial-Directed Enzyme Prodrug Therapy. J. Control. Release 2013, 170 (1), 120–131. [PubMed: 23688772]
55. Wang S-B; Liu X-H; Li B; Fan J-X; Ye J-J; Cheng H; Zhang X-Z, Bacteria-Assisted Selective Photothermal Therapy for Precise Tumor Inhibition. Adv. Funct. Mater 2019, 29 (35), 1904093.

56. Fan JX; Peng MY; Wang H; Zheng HR; Liu ZL; Li CX; Wang XN; Liu XH; Cheng SX; Zhang XZ, Engineered Bacterial Bioreactor for Tumor Therapy *via* Fenton-Like Reaction with Localized H_2O_2 Generation. Adv. Mater 2019, 31 (16), 1808278.
57. Ho CL; Tan HQ; Chua KJ; Kang A; Lim KH; Ling KL; Yew WS; Lee YS; Thiery JP; Chang MW, Engineered Commensal Microbes for Diet-Mediated Colorectal-Cancer Chemoprevention. Nat. Biomed. Eng 2018, 2 (1), 27–37. [PubMed: 31015663]
58. Farjadian F; Moghoofei M; Mirkiani S; Ghasemi A; Rabiee N; Hadifar S; Beyzavi A; Karimi M; Hamblin MR, Bacterial Components as Naturally Inspired Nano-Carriers for Drug/Gene Delivery and Immunization: Set the Bugs to Work? Biotechnol. Adv 2018, 36 (4), 968–985. [PubMed: 29499341]
59. Wang S; Gao J; Wang Z, Outer Membrane Vesicles for Vaccination and Targeted Drug Delivery. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol 2019, 11 (2), e1523. [PubMed: 29701017]
60. Clarke AJ, The "Hole" Story of Predatory Outer-Membrane Vesicles. Can. J. Microbiol 2018, 64 (9), 589–599. [PubMed: 30169125]
61. Li Z; Clarke AJ; Beveridge TJ, A Major Autolysin of *Pseudomonas aeruginosa*: Subcellular Distribution, Potential Role in Cell Growth and Division, and Secretion in Surface Membrane Vesicles. J. Bacteriol 1996, 178 (9), 2479–2488. [PubMed: 8626312]
62. Kadurugamuwa JL; Beveridge TJ, Bacteriolytic Effect of Membrane Vesicles from *Pseudomonas aeruginosa* on Other Bacteria Including Pathogens: Conceptually New Antibiotics. J. Bacteriol 1996, 178 (10), 2767–2774. [PubMed: 8631663]
63. Haurat MF; Aduse-Opoku J; Rangarajan M; Dorobantu L; Gray MR; Curtis MA; Feldman MF, Selective Sorting of Cargo Proteins into Bacterial Membrane Vesicles. J. Biol. Chem 2011, 286 (2), 1269–1276. [PubMed: 21056982]
64. Elhenawy W; Debelyy MO; Feldman MF, Preferential Packing of Acidic Glycosidases and Proteases into *Bacteroides* Outer Membrane Vesicles. mBio 2014, 5 (2), e00909–14. [PubMed: 24618254]
65. Evans AGL; Davey HM; Cookson A; Currinn H; Cooke-Fox G; Stanczyk PJ; Whitworth DE, Predatory Activity of *Myxococcus xanthus* Outer-Membrane Vesicles and Properties of Their Hydrolase Cargo. Microbiology 2012, 158 (Pt 11), 2742–2752. [PubMed: 22977088]
66. Li Z; Clarke AJ; Beveridge TJ, Gram-Negative Bacteria Produce Membrane Vesicles Which Are Capable of Killing Other Bacteria. J. Bacteriol 1998, 180 (20), 5478–5483. [PubMed: 9765585]
67. Gujrati V; Kim S; Kim SH; Min JJ; Choy HE; Kim SC; Jon S, Bioengineered Bacterial Outer Membrane Vesicles as Cell-Specific Drug-Delivery Vehicles for Cancer Therapy. ACS Nano 2014, 8 (2), 1525–1537. [PubMed: 24410085]
68. Fang RH; Jiang Y; Fang JC; Zhang L, Cell Membrane-Derived Nanomaterials for Biomedical Applications. Biomaterials 2017, 128, 69–83. [PubMed: 28292726]
69. Fang RH; Kroll AV; Gao W; Zhang L, Cell Membrane Coating Nanotechnology. Adv. Mater 2018, 30 (23), 1706759.
70. Zhang Y; Chen Y; Lo C; Zhuang J; Angsantikul P; Zhang Q; Wei X; Zhou Z; Obonyo M; Fang RH; Gao W; Zhang L, Inhibition of Pathogen Adhesion by Bacterial Outer Membrane-Coated Nanoparticles. Angew. Chem. Int. Ed. Engl 2019, 58 (33), 11404–11408. [PubMed: 31206942]
71. Gao W; Fang RH; Thamphiwatana S; Luk BT; Li J; Angsantikul P; Zhang Q; Hu C-MJ; Zhang L, Modulating Antibacterial Immunity *via* Bacterial Membrane-Coated Nanoparticles. Nano. Lett 2015, 15 (2), 1403–1409. [PubMed: 25615236]
72. Shao J; Xuan M; Zhang H; Lin X; Wu Z; He Q, Chemotaxis-Guided Hybrid Neutrophil Micromotors for Targeted Drug Transport. Angew. Chem. Int. Ed. Engl 2017, 56 (42), 12935–12939. [PubMed: 28816386]
73. Kolaczkowska E; Kubes P, Neutrophil Recruitment and Function in Health and Inflammation. Nat. Rev. Immunol 2013, 13 (3), 159–175. [PubMed: 23435331]
74. Gao F; Xu L; Yang B; Fan F; Yang L, Kill the Real with the Fake: Eliminate Intracellular *Staphylococcus aureus* using Nanoparticle Coated with Its Extracellular Vesicle Membrane as Active-Targeting Drug Carrier. ACS Infect. Dis 2019, 5 (2), 218–227. [PubMed: 30489062]
75. Fraunholz M; Sinha B, Intracellular *Staphylococcus aureus*: Live-In and Let Die. Front. Cell. Infect. Microbiol 2012, 2, 43. [PubMed: 22919634]

76. Bowdish DME; Loffredo MS; Mukhopadhyay S; Mantovani A; Gordon S, Macrophage Receptors Implicated in the “Adaptive” Form of Innate Immunity. Microbes Infect 2007, 9 (14–15), 1680–1687. [PubMed: 18023392]
77. Mitragotri S; Burke PA; Langer R, Overcoming the Challenges in Administering Biopharmaceuticals: Formulation and Delivery Strategies. Nat. Rev. Drug Discov 2014, 13 (9), 655–672. [PubMed: 25103255]
78. Beilhartz GL; Sugiman-Marangos SN; Melnyk RA, Repurposing Bacterial Toxins for Intracellular Delivery of Therapeutic Proteins. Biochem. Pharmacol 2017, 142, 13–20. [PubMed: 28408344]
79. Liao X; Rabideau AE; Pentelute BL, Delivery of Antibody Mimics into Mammalian Cells *via* Anthrax Toxin Protective Antigen. ChemBioChem 2014, 15 (16), 2458–2466. [PubMed: 25250705]
80. Dyer PDR; Shepherd TR; Gollings AS; Shorter SA; Gorringe-Pattrick MAM; Tang CK; Cattoz BN; Baillie L; Griffiths PC; Richardson SCW, Disarmed Anthrax Toxin Delivers Antisense Oligonucleotides and siRNA with High Efficiency and Low Toxicity. J. Control. Release 2015, 220 (Pt A), 316–328. [PubMed: 26546271]
81. Rabideau AE; Pentelute BL, Delivery of Non-Native Cargo into Mammalian Cells using Anthrax Lethal Toxin. ACS Chem. Biol 2016, 11 (6), 1490–1501. [PubMed: 27055654]
82. Zahaf NI; Schmidt G, Bacterial Toxins for Cancer Therapy. Toxins 2017, 9 (8), 236.
83. Duvic M; Kuzel TM; Olsen EA; Martin AG; Foss FM; Kim YH; Heald PW; Bacha P; Nichols J; Liepa A, Quality-of-Life Improvements in Cutaneous T-Cell Lymphoma Patients Treated with Denileukin Diftitox (ONTAK®). Clin. Lymphoma 2002, 2 (4), 222–228. [PubMed: 11970761]
84. Kreitman J, R.; Pastan I, Antibody Fusion Proteins: Anti-CD22 Recombinant Immunotoxin Moxetumomab Pasudotox. Clin. Cancer Res 2011, 17 (20), 6398–6405. [PubMed: 22003067]
85. Fox ME; Lemmon MJ; Mauchline ML; Davis TO; Giaccia AJ; Minton NP; Brown JM, Anaerobic Bacteria as a Delivery System for Cancer Gene Therapy: *In Vitro* Activation of 5-Fluorocytosine by Genetically Engineered Clostridia. Gene Ther. 1996, 3 (2), 173–178. [PubMed: 8867865]
86. Heap JT; Theys J; Ehsaan M; Kubiak AM; Dubois L; Paesmans K; Van Mellaert L; Knox R; Kuehne SA; Lambin P; Minton NP, Spores of *Clostridium* Engineered for Clinical Efficacy and Safety Cause Regression and Cure of Tumors *In Vivo*. Oncotarget 2014, 5 (7), 1761–1769. [PubMed: 24732092]
87. Song Q; Zheng C; Jia J; Zhao H; Feng Q; Zhang H; Wang L; Zhang Z; Zhang Y, A Probiotic Spore-Based Oral Autonomous Nanoparticles Generator for Cancer Therapy. Adv. Mater 2019, 31 (43), 1903793.
88. Malmgren RA; Flanigan CC, Localization of the Vegetative Form of *Clostridium tetani* in Mouse Tumors following Intravenous Spore Administration. Cancer Res. 1955, 15 (7), 473–478. [PubMed: 13240693]
89. Luo CH; Huang CT; Su CH; Yeh CS, Bacteria-Mediated Hypoxia-Specific Delivery of Nanoparticles for Tumors Imaging and Therapy. Nano. Lett 2016, 16 (6), 3493–3499. [PubMed: 27148804]
90. Claesen J; Fischbach MA, Synthetic Microbes as Drug Delivery Systems. ACS Synth. Biol 2015, 4 (4), 358–364. [PubMed: 25079685]
91. Lemmer Y; Kalombo L; Pietersen RD; Jones AT; Semete-Makokotlela B; Van Wyngaardt S; Ramalapa B; Stoltz AC; Baker B; Verschoor JA; Swai HS; De Chastellier C, Mycolic Acids, a Promising Mycobacterial Ligand for Targeting of Nanoencapsulated Drugs in Tuberculosis. J. Control. Release 2015, 211, 94–104. [PubMed: 26055640]
92. Guo A; Durymanov M; Permyakova A; Sene S; Serre C; Reineke J, Metal Organic Framework (MOF) Particles as Potential Bacteria-Mimicking Delivery Systems for Infectious Diseases: Characterization and Cellular Internalization in Alveolar Macrophages. Pharm. Res 2019, 36 (4), 53. [PubMed: 30790066]
93. Labouta HI; Menina S; Kochut A; Gordon S; Geyer R; Dersch P; Lehr CM, Bacteriomimetic Invasin-Functionalized Nanocarriers for Intracellular Delivery. J. Control. Release 2015, 220 (Pt A), 414–424. [PubMed: 26522071]
94. Lee C; Fraser B; Szu S; Lin K, Chemical Structure of and Immune Response to Polysaccharides of *Streptococcus pneumoniae*. Rev. Infect. Dis 1981, 3 (2), 323–331. [PubMed: 7020047]

95. Vassallo R; Thomas CF; Vuk-Pavlovic Z; Limper AH, Alveolar Macrophage Interactions with *Pneumocystis carinii*. J. Lab. Clin. Med 1999, 133 (6), 535–540. [PubMed: 10360627]

96. Rajaram MVS; Brooks MN; Morris JD; Torrelles JB; Azad AK; Schlesinger LS, *Mycobacterium tuberculosis* Activates Human Macrophage Peroxisome Proliferator-Activated Receptor γ Linking Mannose Receptor Recognition to Regulation of Immune Responses. J. Immunol 2010, 185 (2), 929–942. [PubMed: 20554962]

97. Tottori S; Zhang L; Qiu F; Krawczyk KK; Franco-Obregón A; Nelson BJ, Magnetic Helical Micromachines: Fabrication, Controlled Swimming, and Cargo Transport. Adv. Mater 2012, 24 (6), 811–816. [PubMed: 22213276]

98. Qiu F; Mhanna R; Zhang L; Ding Y; Fujita S; Nelson BJ, Artificial Bacterial Flagella Functionalized with Temperature-Sensitive Liposomes for Controlled Release. Sens. Actuators B Chem 2014, 196, 676–681.

99. Servant A; Qiu F; Mazza M; Kostarelos K; Nelson BJ, Controlled In Vivo Swimming of a Swarm of Bacteria-Like Microrobotic Flagella. Adv. Mater 2015, 27 (19), 2981–2988. [PubMed: 25850420]

100. Serrano P; Decanini D; Leroy L; Couraud L; Hwang G, Multiflagella Artificial Bacteria for Robust Microfluidic Propulsion and Multimodal Micromanipulation. Microelectron. Eng 2018, 195, 145–152.

101. Huang HW; Uslu FE; Katsamba P; Lauga E; Sakar MS; Nelson BJ, Adaptive Locomotion of Artificial Microswimmers. Sci. Adv 2019, 5 (1), eaau1532. [PubMed: 30746446]

102. Schuerle S; Soleimany AP; Yeh T; Anand GM; Häberli M; Fleming HE; Mirkhani N; Qiu F; Hauert S; Wang X; Nelson BJ; Bhatia SN, Synthetic and Living Micropropellers for Convection-Enhanced Nanoparticle Transport. Sci. Adv 2019, 5 (4), eaav4803. [PubMed: 31032412]

103. Mogensen TH, Pathogen Recognition and Inflammatory Signaling in Innate Immune Defenses. Clin. Microbiol. Rev 2009, 22 (2), 240–273. [PubMed: 19366914]

104. Zheng JH; Nguyen VH; Jiang S-N; Park S-H; Tan W; Hong SH; Shin MG; Chung I-J; Hong Y; Bom H-S; Choy HE; Lee SE; Rhee JH; Min J-J, Two-Step Enhanced Cancer Immunotherapy with Engineered *Salmonella* Typhimurium Secreting Heterologous Flagellin. Sci. Transl. Med 2017, 9 (376), eaak9537. [PubMed: 28179508]

105. Zepp F, Principles of Vaccine Design—Lessons from Nature. Vaccine 2010, 28 (Suppl 3), C14–C24. [PubMed: 20713252]

106. Fang RH; Kroll AV; Zhang L, Nanoparticle-Based Manipulation of Antigen-Presenting Cells for Cancer Immunotherapy. Small 2015, 11 (41), 5483–5496. [PubMed: 26331993]

107. Zhou J; Kroll AV; Holay M; Fang RH; Zhang L, Biomimetic Nanotechnology toward Personalized Vaccines. Adv. Mater 2020, 32 (13), 1901255.

108. Kroll AV; Jiang Y; Zhou J; Holay M; Fang RH; Zhang L, Biomimetic Nanoparticle Vaccines for Cancer Therapy. Adv. Biosyst 2019, 3 (1), 1800219.

109. Tang J; Shalabi A; Hubbard-Lucey VM, Comprehensive Analysis of the Clinical Immuno-Oncology Landscape. Ann. Oncol 2018, 29 (1), 84–91. [PubMed: 29228097]

110. Zhang H; Chen J, Current Status and Future Directions of Cancer Immunotherapy. J. Cancer 2018, 9 (10), 1773–1781. [PubMed: 29805703]

111. Gong J; Chehrazi-raffle A; Reddi S; Salgia R, Development of PD-1 and PD-L1 Inhibitors as a Form of Cancer Immunotherapy: A Comprehensive Review of Registration Trials and Future Considerations. J. Immunother. Cancer 2018, 6, 8. [PubMed: 29357948]

112. Pitt JM; Vetizou M; Daillere R; Roberti MP; Yamazaki T; Routy B; Lepage P; Boneca IG; Chamaillard M; Kroemer G; Zitvogel L, Resistance Mechanisms to Immune-Checkpoint Blockade in Cancer: Tumor-Intrinsic and -Extrinsic Factors. Immunity 2016, 44 (6), 1255–1269. [PubMed: 27332730]

113. Forbes NS; Coffin RS; Deng L; Evgin L; Fiering S; Giacalone M; Gravekamp C; Gulley JL; Gunn H; Hoffman RM; Kaur B; Liu K; Lyerly HK; Marciscano AE; Moradian E; Ruppel S; Saltzman DA; Tattersall PJ; Thorne S; Vile RG; Zhang HH; Zhou S; McFadden G, White Paper on Microbial Anti-Cancer Therapy and Prevention. J. Immunother. Cancer 2018, 6, 78. [PubMed: 30081947]

114. Zhang Y; Luo F; Li A; Qian J; Yao Z; Feng X; Chu Y, Systemic Injection of TLR1/2 Agonist Improves Adoptive Antigen-Specific T Cell Therapy in Glioma-Bearing Mice. Clin. Immunol 2014, 154 (1), 26–36. [PubMed: 24928324]

115. Grille S; Moreno M; Bascuas T; Marqués JM; Muñoz N; Lens D; Chabalgoity JA, *Salmonella enterica* Serovar Typhimurium Immunotherapy for B-Cell Lymphoma Induces Broad Anti-Tumour Immunity with Therapeutic Effect. Immunology 2014, 143 (3), 428–437. [PubMed: 24834964]

116. Richer MJ; Nolz JC; Harty JT, Pathogen-Specific Inflammatory Milieux Tune the Antigen Sensitivity of $CD8^+$ T Cells by Enhancing T Cell Receptor Signaling. Immunity 2013, 38 (1), 140–152. [PubMed: 23260194]

117. Kalupahana RS; Mastroeni P; Maskell D; Blacklaws BA, Activation of Murine Dendritic Cells and Macrophages Induced by *Salmonella enterica* Serovar Typhimurium. Immunology 2005, 115 (4), 462–472. [PubMed: 16011515]

118. Bellora F; Castriconi R; Dondero A; Pessino A; Nencioni A; Liggieri G; Moretta L; Mantovani A; Moretta A; Bottino C, TLR Activation of Tumor-Associated Macrophages from Ovarian Cancer Patients Triggers Cytolytic Activity of NK Cells. Eur. J. Immunol 2014, 44 (6), 1814–1822. [PubMed: 24510590]

119. Zhang Y; Luo F; Cai Y; Liu N; Wang L; Xu D; Chu Y, TLR1/TLR2 Agonist Induces Tumor Regression by Reciprocal Modulation of Effector and Regulatory T Cells. J. Immunol 2011, 186 (4), 1963–1969. [PubMed: 21217015]

120. Zheng JH; Min J-J, Targeted Cancer Therapy using Engineered *Salmonella* Typhimurium. Chonnam Med. J 2016, 52 (3), 173–184. [PubMed: 27689027]

121. Roberts NJ; Zhang L; Janku F; Collins A; Bai RY; Staedtke V; Rusk AW; Tung D; Miller M; Roix J; Khanna KV; Murthy R; Benjamin RS; Helgason T; Szvalb AD; Bird JE; Roy-Chowdhuri S; Zhang HH; Qiao Y; Karim B; McDaniel J; Elpiner A; Sahora A; Lachowicz J; Phillips B; Turner A; Klein MK; Post G; Diaz LA Jr.; Riggins GJ; Papadopoulos N; Kinzler KW; Vogelstein B; Bettegowda C; Huso DL; Varterasian M; Saha S; Zhou S, Intratumoral Injection of *Clostridium novyi*-NT Spores Induces Antitumor Responses. Sci. Transl. Med 2014, 6 (249), 249ra111.

122. Wilkinson EM; Ilhan ZE; Herbst-Kralovetz MM, Microbiota-Drug Interactions: Impact on Metabolism and Efficacy of Therapeutics. Maturitas 2018, 112, 53–63. [PubMed: 29704918]

123. Pilgrim S; Stritzker J; Schoen C; Kolb-Mäurer A; Geginat G; Loessner MJ; Gentschev I; Goebel W, Bactofection of Mammalian Cells by *Listeria monocytogenes*: Improvement and Mechanism of DNA Delivery. Gene Ther 2003, 10 (24), 2036–2045. [PubMed: 14566363]

124. Toussaint B; Chauchet X; Wang Y; Polack B; Gouëllec AL, Live-Attenuated Bacteria as a Cancer Vaccine Vector. Expert Rev. Vaccines 2013, 12 (10), 1139–1154. [PubMed: 24124876]

125. Kim OY; Park HT; Dinh NTH; Choi SJ; Lee J; Kim JH; Lee S-W; Gho YS, Bacterial Outer Membrane Vesicles Suppress Tumor by Interferon-γ-Mediated Antitumor Response. Nat. Commun 2017, 8, 626. [PubMed: 28931823]

126. Chen Q; Bai H; Wu W; Huang G; Li Y; Wu M; Tang G; Ping Y, Bioengineering Bacterial Vesicle-Coated Polymeric Nanomedicine for Enhanced Cancer Immunotherapy and Metastasis Prevention. Nano. Lett 2020, 20 (1), 11–21. [PubMed: 31858807]

127. Nallar SC; Xu DQ; Kalvakolanu DV, Bacteria and Genetically Modified Bacteria as Cancer Therapeutics: Current Advances and Challenges. Cytokine 2017, 89, 160–172. [PubMed: 26778055]

128. Zheng B; Xu J; Chen G; Zhang S; Xiao Z; Lu W, Bacterium-Mimicking Vector with Enhanced Adjuvanticity for Cancer Immunotherapy and Minimized Toxicity. Adv. Funct. Mater 2019, 29 (33), 1901437.

129. Ding C; Ma J; Dong Q; Liu Q, Live Bacterial Vaccine Vector and Delivery Strategies of Heterologous Antigen: A Review. Immunol. Lett 2018, 197, 70–77. [PubMed: 29550258]

130. Michon C; Langella P; Eijsink VGH; Mathiesen G; Chatel JM, Display of Recombinant Proteins at the Surface of Lactic Acid Bacteria: Strategies and Applications. Microb. Cell Fact 2016, 15, 70. [PubMed: 27142045]

131. Flickinger JC Jr.; Rodeck U; Snook AE, *Listeria monocytogenes* as a Vector for Cancer Immunotherapy: Current Understanding and Progress. Vaccines 2018, 6 (3), 48.

132. Ni D; Qing S; Ding H; Yue H; Yu D; Wang S; Luo N; Su Z; Wei W; Ma G, Biomimetically Engineered Demi-Bacteria Potentiate Vaccination against Cancer. Adv. Sci 2017, 4 (10), 1700083.
133. Langemann T; Koller VJ; Muhammad A; Kudela P; Mayr UB; Lubitz W, The Bacterial Ghost Platform System. Bioeng. Bugs 2010, 1 (5), 326–336. [PubMed: 21326832]
134. Michalek J; Hezova R; Turanek-Knötigova P; Gabkova J; Strioga M; Lubitz W; Kudela P, Oncolysate-Loaded *Escherichia coli* Bacterial Ghosts Enhance the Stimulatory Capacity of Human Dendritic Cells. Cancer Immunol. Immunother 2017, 66 (2), 149–159. [PubMed: 27864613]
135. Dobrovolskiene N; Pasukoniene V; Darinskas A; Krasko JA; Zilionyte K; Mlynska A; Gudleviciene Z; Miseikyte-Kaubriene E; Schijns V; Lubitz W; Kudela P; Strioga M, Tumor Lysate-Loaded Bacterial Ghosts as a Tool for Optimized Production of Therapeutic Dendritic Cell-Based Cancer Vaccines. Vaccine 2018, 36 (29), 4171–4180. [PubMed: 29895501]
136. Zhang Y; Fang Z; Li R; Huang X; Liu Q, Design of Outer Membrane Vesicles as Cancer Vaccines: A New Toolkit for Cancer Therapy. Cancers 2019, 11 (9), 1314.
137. Wang S; Huang W; Li K; Yao Y; Yang X; Bai H; Sun W; Liu C; Ma Y, Engineered Outer Membrane Vesicle is Potent to Elicit HPV16E7-Specific Cellular Immunity in a Mouse Model of TC-1 Graft Tumor. Int. J. Nanomed 2017, 12, 6813–6825.
138. Chen Q; Huang G; Wu W; Wang J; Hu J; Mao J; Chu PK; Bai H; Tang G, A Hybrid Eukaryotic–Prokaryotic Nanoplatform with Photothermal Modality for Enhanced Antitumor Vaccination. Adv. Mater 2020, 32 (16), 1908185.
139. Patel RB; Ye M; Carlson PM; Jaquish A; Zangl L; Ma B; Wang Y; Arthur I; Xie R; Brown RJ; Wang X; Sriramaneni R; Kim K; Gong S; Morris ZS, Development of an *In Situ* Cancer Vaccine *via* Combinational Radiation and Bacterial-Membrane-Coated Nanoparticles. Adv. Mater 2019, 31 (43), 1902626.
140. Chanier T; Chames P, Nanobody Engineering: Toward Next Generation Immunotherapies and Immunoimaging of Cancer. Antibodies 2019, 8 (1), 13.
141. Gurbatri CR; Lia I; Vincent R; Coker C; Castro S; Treuting PM; Hinchliffe TE; Arpaia N; Danino T, Engineered Probiotics for Local Tumor Delivery of Checkpoint Blockade Nanobodies. Sci. Transl. Med 2020, 12 (530), eaax0876. [PubMed: 32051224]
142. Oldenborg PA; Zheleznyak A; Fang YF; Lagenaur CF; Gresham HD; Lindberg FP, Role of CD47 as a Marker of Self on Red Blood Cells. Science 2000, 288 (5473), 2051–2054. [PubMed: 10856220]
143. Advani R; Flinn I; Popplewell L; Forero A; Bartlett NL; Ghosh N; Kline J; Roschewski M; LaCasce A; Collins GP; Tran T; Lynn J; Chen JY; Volkmer JP; Agoram B; Huang J; Majeti R; Weissman IL; Takimoto CH; Chao MP; Smith SM, CD47 Blockade by Hu5F9-G4 and Rituximab in Non-Hodgkin's Lymphoma. N. Engl. J. Med 2018, 379 (18), 1711–1721. [PubMed: 30380386]
144. Ingram JR; Blomberg OS; Sockolosky JT; Ali L; Schmidt FI; Pishesha N; Espinosa C; Dougan SK; Garcia KC; Ploegh HL; Dougan M, Localized CD47 Blockade Enhances Immunotherapy for Murine Melanoma. Proc. Natl. Acad. Sci. U. S. A 2017, 114 (38), 10184–10189. [PubMed: 28874561]
145. Chowdhury S; Castro S; Coker C; Hinchliffe TE; Arpaia N; Danino T, Programmable Bacteria Induce Durable Tumor Regression and Systemic Antitumor Immunity. Nat. Med 2019, 25 (7), 1057–1063. [PubMed: 31270504]
146. Griesenauer RH; Kinch MS, An Overview of FDA-Approved Vaccines & Their Innovators. Expert Rev. Vaccines 2017, 16 (12), 1253–1266. [PubMed: 28931331]
147. Lindberg AA, The History of Live Bacterial Vaccines. Dev. Biol. Stand 1995, 84, 211–219. [PubMed: 7796956]
148. Minor PD, Live Attenuated Vaccines: Historical Successes and Current Challenges. Virology 2015, 479–480, 379–392. [PubMed: 25864107]
149. Gentschev I; Spreng S; Sieber H; Ures J; Mollet F; Collioud A; Pearman J; Griot-Wenk ME; Fensterle J; Rapp UR, Vivotif®–A 'Magic Shield' for Protection against Typhoid Fever and Delivery of Heterologous Antigens. Chemotherapy 2007, 53 (3), 177–180. [PubMed: 17347563]

150. Plotkin S, History of Vaccination. Proc. Natl. Acad. Sci. U. S. A 2014, 111 (34), 12283–12287. [PubMed: 25136134]

151. Kapil P; Merkel TJ, Pertussis Vaccines and Protective Immunity. Curr. Opin. Immunol 2019, 59, 72–78. [PubMed: 31078081]

152. Granoff DM; Rathore MH; Holmes SJ; Granoff PD; Lucas AH, Effect of Immunity to the Carrier Protein on Antibody Responses to *Haemophilus influenzae* Type B Conjugate Vaccines. Vaccine 1993, 11 (Suppl 1), S46–S51. [PubMed: 8447176]

153. Irvine DJ, Materializing the Future of Vaccines and Immunotherapy. Nat. Rev. Mater 2016, 1 (1), 15008.

154. Kamei A; Coutinho-Sledge YS; Goldberg JB; Priebe GP; Pier GB, Mucosal Vaccination with a Multivalent, Live-Attenuated Vaccine Induces Multifactorial Immunity against *Pseudomonas aeruginosa* Acute Lung Infection. Infect. Immun 2011, 79 (3), 1289–1299. [PubMed: 21149583]

155. Kaushal D; Foreman TW; Gautam US; Alvarez X; Adekambi T; Rangel-Moreno J; Golden NA; Johnson AMF; Phillips BL; Ahsan MH; Russell-Lodrigue KE; Doyle LA; Roy CJ; Didier PJ; Blanchard JL; Rengarajan J; Lackner AA; Khader SA; Mehra S, Mucosal Vaccination with Attenuated *Mycobacterium tuberculosis* Induces Strong Central Memory Responses and Protects against Tuberculosis. Nat. Commun 2015, 6, 8533. [PubMed: 26460802]

156. Cabral MP; Garcia P; Beceiro A; Rumbo C; Perez A; Moscoso M; Bou G, Design of Live Attenuated Bacterial Vaccines Based on D-Glutamate Auxotrophy. Nat. Commun 2017, 8, 15480. [PubMed: 28548079]

157. Jawale CV; Lee JH, Comparative Evaluation of *Salmonella* Enteritidis Ghost Vaccines with a Commercial Vaccine for Protection against Internal Egg Contamination with *Salmonella*. Vaccine 2014, 32 (45), 5925–5930. [PubMed: 25218296]

158. Hajam IA; Dar PA; Appavoo E; Kishore S; Bhanuprakash V; Ganesh K, Bacterial Ghosts of *Escherichia coli* Drive Efficient Maturation of Bovine Monocyte-Derived Dendritic Cells. PLOS ONE 2015, 10 (12), e0144397. [PubMed: 26669936]

159. Hajam IA; Dar PA; Won G; Lee JH, Bacterial Ghosts as Adjuvants: Mechanisms and Potential. Vet. Res 2017, 48, 37. [PubMed: 28645300]

160. Muhammad A; Kassmannhuber J; Rauscher M; Falcon AA; Wheeler DW; Zhang AA; Lubitz P; Lubitz W, Subcutaneous Immunization of Dogs with Bordetella bronchiseptica Bacterial Ghost Vaccine. Front. Immunol 2019, 10, 1377. [PubMed: 31293571]

161. Lim JL; Koh VHQ; Cho SSL; Periaswamy B; Choi DPS; Vacca M; De Sessions PF; Kudela P; Lubitz W; Pastorin G; Alonso S, Harnessing the Immunomodulatory Properties of Bacterial Ghosts to Boost the Anti-Mycobacterial Protective Immunity. Front. Immunol 2019, 10, 2737. [PubMed: 31824511]

162. Schwechheimer C; Kuehn MJ, Outer-Membrane Vesicles from Gram-Negative Bacteria: Biogenesis and Functions. Nat. Rev. Microbiol 2015, 13 (10), 605–619. [PubMed: 26373371]

163. Scorza FB; Doro F; Rodríguez-Ortega MJ; Stella M; Liberatori S; Taddei AR; Serino L; Moriel DG; Nesta B; Fontana MR, Proteomics Characterization of Outer Membrane Vesicles from the Extraintestinal Pathogenic *Escherichia coli* ΔtolR IHE3034 Mutant. Mol. Cell. Proteomics 2008, 7 (3), 473–485. [PubMed: 17982123]

164. Gorringe AR; Pajón R, Bexsero: A Multicomponent Vaccine for Prevention of Meningococcal Disease. Hum. Vaccines Immunother 2012, 8 (2), 174–183.

165. Holst J; Martin D; Arnold R; Huergo CC; Oster P; O'Hallahan J; Rosenqvist E, Properties and Clinical Performance of Vaccines Containing Outer Membrane Vesicles from *Neisseria meningitidis*. Vaccine 2009, 27 (Suppl 2), B3–B12. [PubMed: 19481313]

166. Van de Waterbeemd B; Streefland M; Van der Ley P; Zomer B; Van Dijken H; Martens D; Wijffels R; Van der Pol L, Improved OMV Vaccine against *Neisseria meningitidis* using Genetically Engineered Strains and a Detergent-Free Purification Process. Vaccine 2010, 28 (30), 4810–4816. [PubMed: 20483197]

167. Keiser P; Biggs-Cicatelli S; Moran E; Schmiel D; Pinto V; Burden R; Miller L; Moon J; Bowden R; Cummings J, A Phase 1 Study of a Meningococcal Native Outer Membrane Vesicle Vaccine Made from a Group B Strain with Deleted *lpxL1* and *synX*, Over-Expressed Factor H Binding

Protein, Two PorAs and Stabilized OpcA Expression. Vaccine 2011, 29 (7), 1413–1420. [PubMed: 21199704]

168. Chen DJ; Osterrieder N; Metzger SM; Buckles E; Doody AM; DeLisa MP; Putnam D, Delivery of Foreign Antigens by Engineered Outer Membrane Vesicle Vaccines. Proc. Natl. Acad. Sci. U. S. A 2010, 107 (7), 3099–3104. [PubMed: 20133740]
169. Huang W; Wang S; Yao Y; Xia Y; Yang X; Li K; Sun P; Liu C; Sun W; Bai H, Employing *Escherichia coli*-Derived Outer Membrane Vesicles as an Antigen Delivery Platform Elicits Protective Immunity against *Acinetobacter baumannii* Infection. Sci. Rep 2016, 6, 37242. [PubMed: 27849050]
170. Salverda ML; Meinderts SM; Hamstra H-J; Wagemakers A; Hovius JW; van der Ark A; Stork M; van der Ley P, Surface Display of a Borrelial Lipoprotein on Meningococcal Outer Membrane Vesicles. Vaccine 2016, 34 (8), 1025–1033. [PubMed: 26801064]
171. Fantappiè L; de Santis M; Chiarot E; Carboni F; Bensi G; Jousson O; Margarit I; Grandi G, Antibody-Mediated Immunity Induced by Engineered *Escherichia coli* OMVs Carrying Heterologous Antigens in their Lumen. J. Extracell. Vesicles 2014, 3, 24015.
172. Camacho A; Irache J; De Souza J; Sánchez-Gómez S; Gamazo C, Nanoparticle-Based Vaccine for Mucosal Protection against *Shigella flexneri* in Mice. Vaccine 2013, 31 (32), 3288–3294. [PubMed: 23727423]
173. Matías J; Brotons A; Cenoz S; Pérez I; Abdulkarim M; Gumbleton M; Irache JM; Gamazo C, Oral Immunogenicity in Mice and Sows of Enterotoxigenic *Escherichia coli* Outer-Membrane Vesicles Incorporated into Zein-Based Nanoparticles. Vaccines 2020, 8 (1), 11.
174. Angsantikul P; Thamphiwatana S; Gao W; Zhang L, Cell Membrane-Coated Nanoparticles as an Emerging Antibacterial Vaccine Platform. Vaccines 2015, 3 (4), 814–828. [PubMed: 26457720]
175. Wu G; Ji H; Guo X; Li Y; Ren T; Dong H; Liu J; Liu Y; Shi X; He B, Nanoparticle Reinforced Bacterial Outer-Membrane Vesicles Effectively Prevent Fatal Infection of Carbapenem-Resistant *Klebsiella pneumoniae*. Nanomedicine 2020, 24, 102148. [PubMed: 31887427]
176. Li M; Zhou H; Yang C; Wu Y; Zhou X; Liu H; Wang Y, Bacterial Outer Membrane Vesicles as a Platform for Biomedical Applications: An Update. J. Control. Release 2020, 323, 253–268. [PubMed: 32333919]
177. Kitchin NR, Review of Diphtheria, Tetanus and Pertussis Vaccines in Clinical Development. Expert Rev. Vaccines 2011, 10 (5), 605–615. [PubMed: 21604982]
178. Cryz S; Fürer E; Germanier R, Effect of Chemical and Heat Inactivation on the Antigenicity and Immunogenicity of *Vibrio cholerae*. Infect. Immun 1982, 38 (1), 21–26. [PubMed: 7141690]
179. Jones RG; Liu Y; Rigsby P; Sesardic D, An Improved Method for Development of Toxoid Vaccines and Antitoxins. J. Immunol. Methods 2008, 337 (1), 42–48. [PubMed: 18571196]
180. Donald RG; Flint M; Kalyan N; Johnson E; Witko SE; Kotash C; Zhao P; Megati S; Yurgelonis I; Lee PK, A Novel Approach to Generate a Recombinant Toxoid Vaccine against *Clostridium difficile*. Microbiology 2013, 159 (Pt 7), 1254–1266. [PubMed: 23629868]
181. Mohammadpour Dounighi N; Eskandari R; Avadi MR; Zolfagharian H; Mir Mohammad Sadeghi A; Rezayat M, Preparation and *In Vitro* Characterization of Chitosan Nanoparticles Containing *Mesobuthus eupeus* Scorpion Venom as an Antigen Delivery System. J. Venom. Anim. Toxins Incl. Trop. Dis 2012, 18 (1), 44–52.
182. Bruno C; Agnolon V; Berti F; Bufali S; O'Hagan DT; Baudner BC, The Preparation and Characterization of PLG Nanoparticles with an Entrapped Synthetic TLR7 Agonist and Their Preclinical Evaluation as Adjuvant for an Adsorbed DTaP Vaccine. Eur. J. Pharm. Biopharm 2016, 105, 1–8. [PubMed: 27224856]
183. Sharma S; Mukkur TK; Benson HA; Chen Y, Enhanced Immune Response against Pertussis Toxoid by IgA-Loaded Chitosan–Dextran Sulfate Nanoparticles. J. Pharm. Sci 2012, 101 (1), 233–244. [PubMed: 21953499]
184. Hu C-MJ; Fang RH; Luk BT; Zhang L, Nanoparticle-Detained Toxins for Safe and Effective Vaccination. Nat. Nanotechnol 2013, 8 (12), 933–938. [PubMed: 24292514]
185. Fang RH; Luk BT; Hu CM; Zhang L, Engineered Nanoparticles Mimicking Cell Membranes for Toxin Neutralization. Adv. Drug Deliv. Rev 2015, 90, 69–80. [PubMed: 25868452]

186. Hu C-MJ; Zhang L, Nanotoxoid Vaccines. Nano Today 2014, 9 (4), 401–404. [PubMed: 25285152]

187. Wang F; Fang RH; Luk BT; Hu CMJ; Thamphiwatana S; Dehaini D; Angsantikul P; Kroll AV; Pang Z; Gao W, Nanoparticle-Based Antivirulence Vaccine for the Management of Methicillin-Resistant *Staphylococcus aureus* Skin Infection. Adv. Funct. Mater 2016, 26 (10), 1628–1635. [PubMed: 27325913]

188. Wei X; Gao J; Wang F; Ying M; Angsantikul P; Kroll AV; Zhou J; Gao W; Lu W; Fang RH, *In Situ* Capture of Bacterial Toxins for Antivirulence Vaccination. Adv. Mater 2017, 29 (33), 1701644.

189. Wei X; Ran D; Campeau A; Xiao C; Zhou J; Dehaini D; Jiang Y; Kroll AV; Zhang Q; Gao W, Multiantigenic Nanotoxoids for Antivirulence Vaccination against Antibiotic-Resistant Gram-Negative Bacteria. Nano. Lett 2019, 19 (7), 4760–4769. [PubMed: 31184899]

190. Wei X; Beltrán-Gastélum M; Karshalev E; Esteban-Fernández de Ávila B; Zhou J; Ran D; Angsantikul P; Fang RH; Wang J; Zhang L, Biomimetic Micromotor Enables Active Delivery of Antigens for Oral Vaccination. Nano. Lett 2019, 19 (3), 1914–1921. [PubMed: 30724085]

191. Angsantikul P; Fang RH; Zhang L, Toxoid Vaccination against Bacterial Infection using Cell Membrane-Coated Nanoparticles. Bioconjug. Chem 2017, 29 (3), 604–612. [PubMed: 29241006]

192. Jiang Y; Krishnan N; Zhou J; Chekuri S; Wei X; Kroll AV; Yu CL; Duan Y; Gao W; Fang RH; Zhang L, Engineered Cell-Membrane-Coated Nanoparticles Directly Present Tumor Antigens to Promote Anticancer Immunity. Adv. Mater 2020, 32 (30), 2001808.

193. Kroll AV; Fang RH; Zhang L, Biointerfacing and Applications of Cell Membrane-Coated Nanoparticles. Bioconjug. Chem 2017, 28 (1), 23–32. [PubMed: 27798829]

194. Zhang Q; Dehaini D; Zhang Y; Zhou J; Chen X; Zhang L; Fang RH; Gao W; Zhang L, Neutrophil Membrane-Coated Nanoparticles Inhibit Synovial Inflammation and Alleviate Joint Damage in Inflammatory Arthritis. Nat. Nanotechnol 2018, 13 (12), 1182–1190. [PubMed: 30177807]

195. Thamphiwatana S; Angsantikul P; Escajadillo T; Zhang Q; Olson J; Luk BT; Zhang S; Fang RH; Gao W; Nizet V; Zhang L, Macrophage-Like Nanoparticles Concurrently Absorbing Endotoxins and Proinflammatory Cytokines for Sepsis Management. Proc. Natl. Acad. Sci. U. S. A 2017, 114 (43), 11488–11493. [PubMed: 29073076]

196. Hu CM; Fang RH; Wang KC; Luk BT; Thamphiwatana S; Dehaini D; Nguyen P; Angsantikul P; Wen CH; Kroll AV; Carpenter C; Ramesh M; Qu V; Patel SH; Zhu J; Shi W; Hofman FM; Chen TC; Gao W; Zhang K; Chien S; Zhang L, Nanoparticle Biointerfacing by Platelet Membrane Cloaking. Nature 2015, 526 (7571), 118–121. [PubMed: 26374997]

197. Fang RH; Hu CM; Luk BT; Gao W; Copp JA; Tai Y; O'Connor DE; Zhang L, Cancer Cell Membrane-Coated Nanoparticles for Anticancer Vaccination and Drug Delivery. Nano. Lett 2014, 14 (4), 2181–2188. [PubMed: 24673373]

198. Zhang Q; Honko A; Zhou J; Gong H; Downs SN; Vasquez JH; Fang RH; Gao W; Griffiths A; Zhang L, Cellular Nanosponges Inhibit SARS-CoV-2 Infectivity. Nano. Lett 2020, 20 (7), 5570–5574. [PubMed: 32551679]

199. Wei X; Zhang G; Ran D; Krishnan N; Fang RH; Gao W; Spector SA; Zhang L, T-Cell-Mimicking Nanoparticles Can Neutralize HIV Infectivity. Adv. Mater 2018, 30 (45), 1802233.

200. Piraner DI; Abedi MH; Moser BA; Lee-Gosselin A; Shapiro MG, Tunable Thermal Bioswitches for *In Vivo* Control of Microbial Therapeutics. Nat. Chem. Biol 2017, 13 (1), 75–80. [PubMed: 27842069]

201. Mandell DJ; Lajoie MJ; Mee MT; Takeuchi R; Kuznetsov G; Norville JE; Gregg CJ; Stoddard BL; Church GM, Biocontainment of Genetically Modified Organisms by Synthetic Protein Design. Nature 2015, 518 (7537), 55–60. [PubMed: 25607366]

202. Rovner AJ; Haimovich AD; Katz SR; Li Z; Grome MW; Gassaway BM; Amiram M; Patel JR; Gallagher RR; Rinehart J; Isaacs FJ, Recoded Organisms Engineered to Depend on Synthetic Amino Acids. Nature 2015, 518 (7537), 89–93. [PubMed: 25607356]

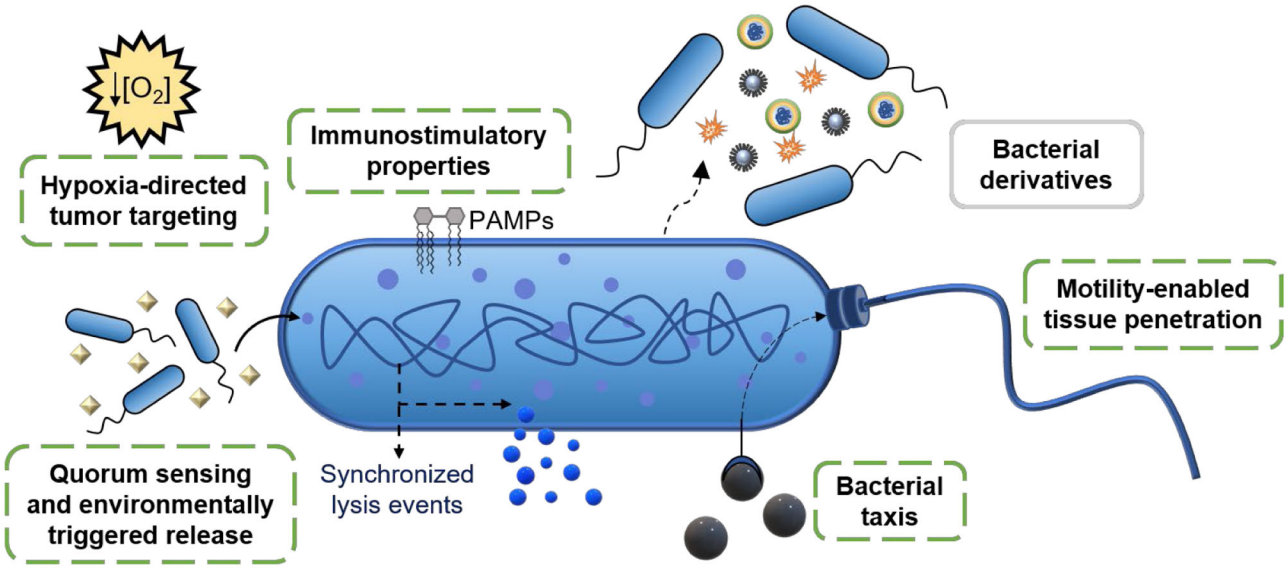


Figure 1. Engineering design principles inspired by bacteria and their derivatives.

The inherent properties of bacteria, including their quorum sensing, environmental sensitivity, taxis, motility, hypoxic growth conditions, and capacity for immune modulation have been leveraged in the design of novel nanomedicine platforms.

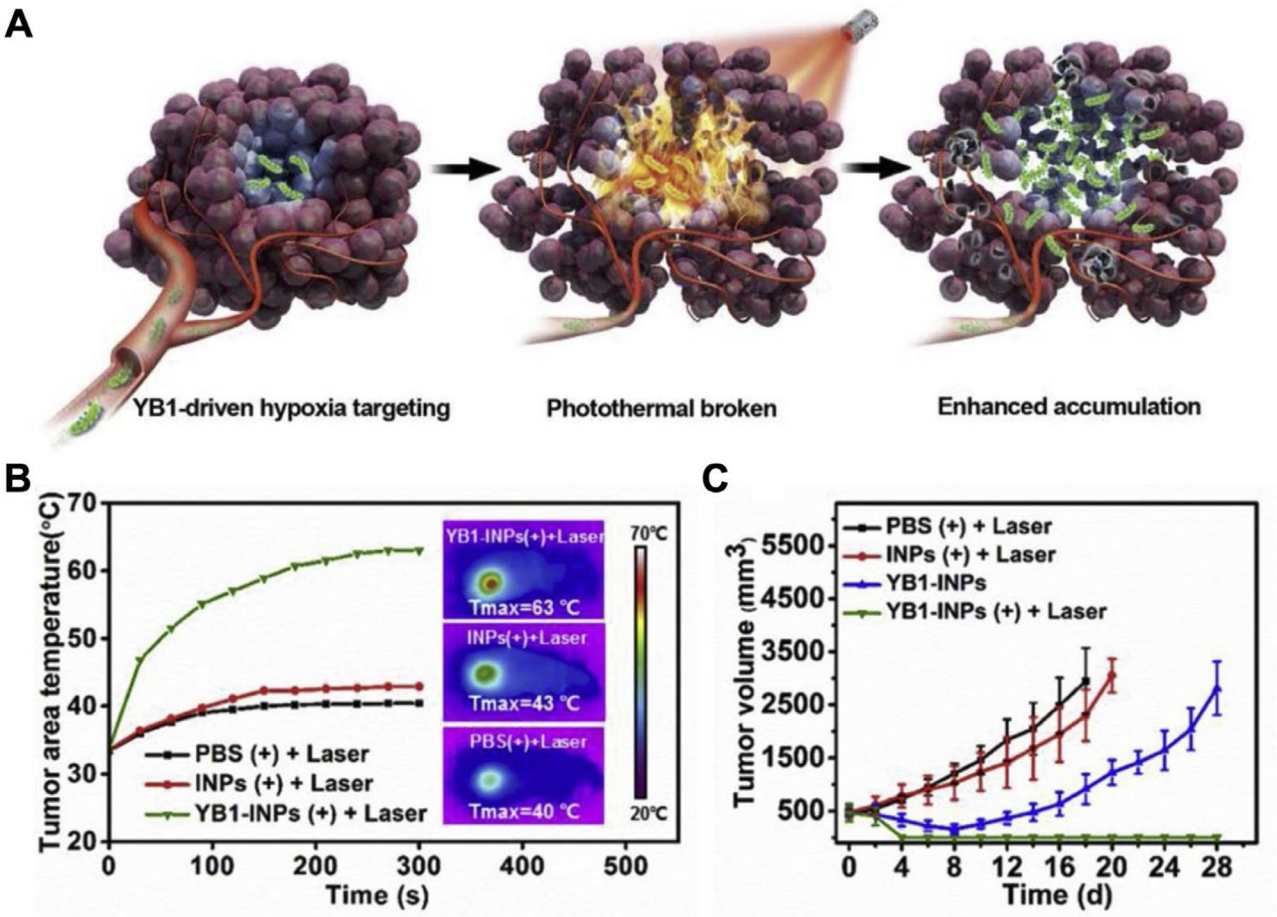


Figure 2. Nanoparticle-modified bacteria for targeted cancer therapy.

(A) *Salmonella* Typhimurium YB1 exhibited enhanced accumulation in tumors due to local hypoxic conditions and the release of bacteria-attracting nutrients post-photothermal therapy. (B) Mice treated with YB1 attached with indocyanine green-loaded nanoparticles (YB1-INPs) showed an elevated temperature in the tumor area upon laser irradiation. (C) Treatment using YB1-INPs combined with laser irradiation led to complete control of MB49 tumor growth in mice. Adapted with permission.[34] Copyright 2019 Elsevier.

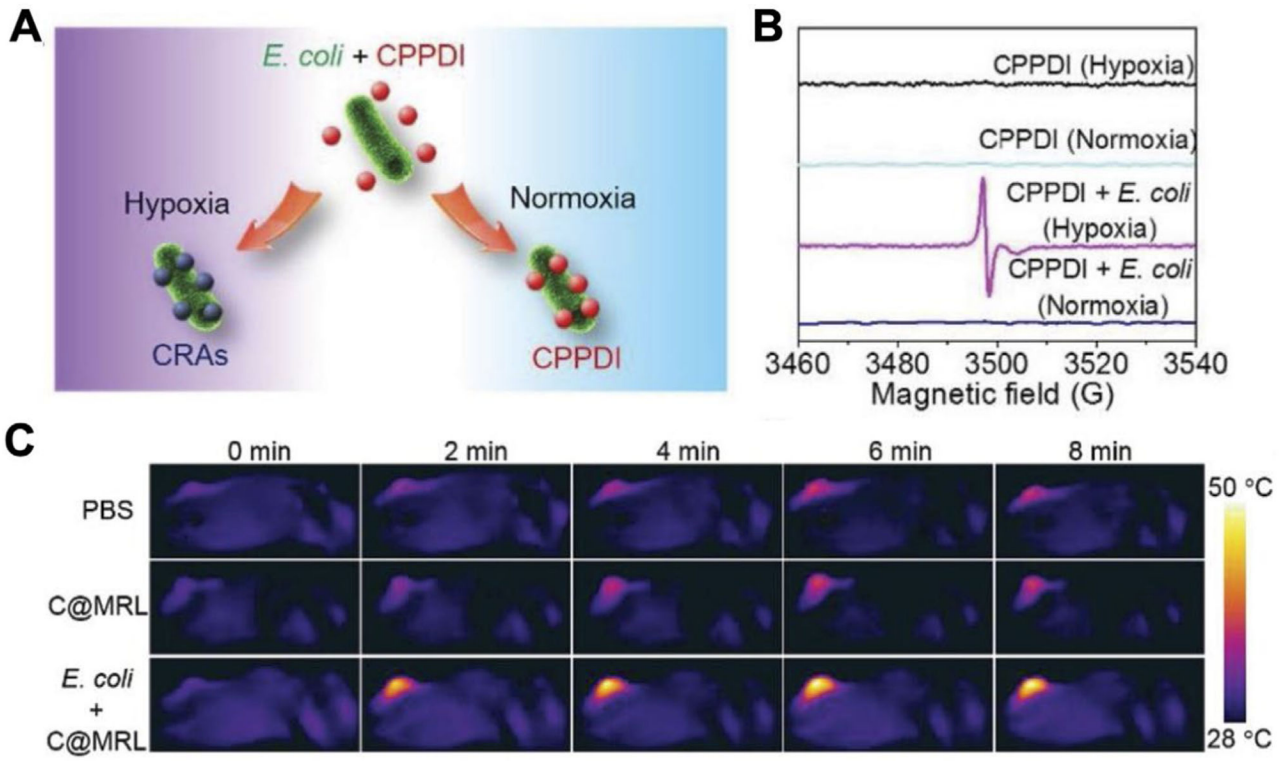


Figure 3. Bacterial bioreactors for localized photothermal therapy.

(A) Under hypoxic conditions, *E. coli* can mediate the reduction of a perylene diimide derivative-based supramolecular complex (CCPDI) into radical anions (CRAs). (B) Electron paramagnetic resonance spectroscopy was used to confirm the selective reduction of CCPDI into CRA in the presence of *E. coli* under hypoxic conditions. (C) The local tumor temperature notably increased in mice administered with *E. coli* and CPPDI-loaded matrix metalloproteinase-2-responsive liposomes (C@MRL). Adapted with permission.[55] Copyright 2019 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

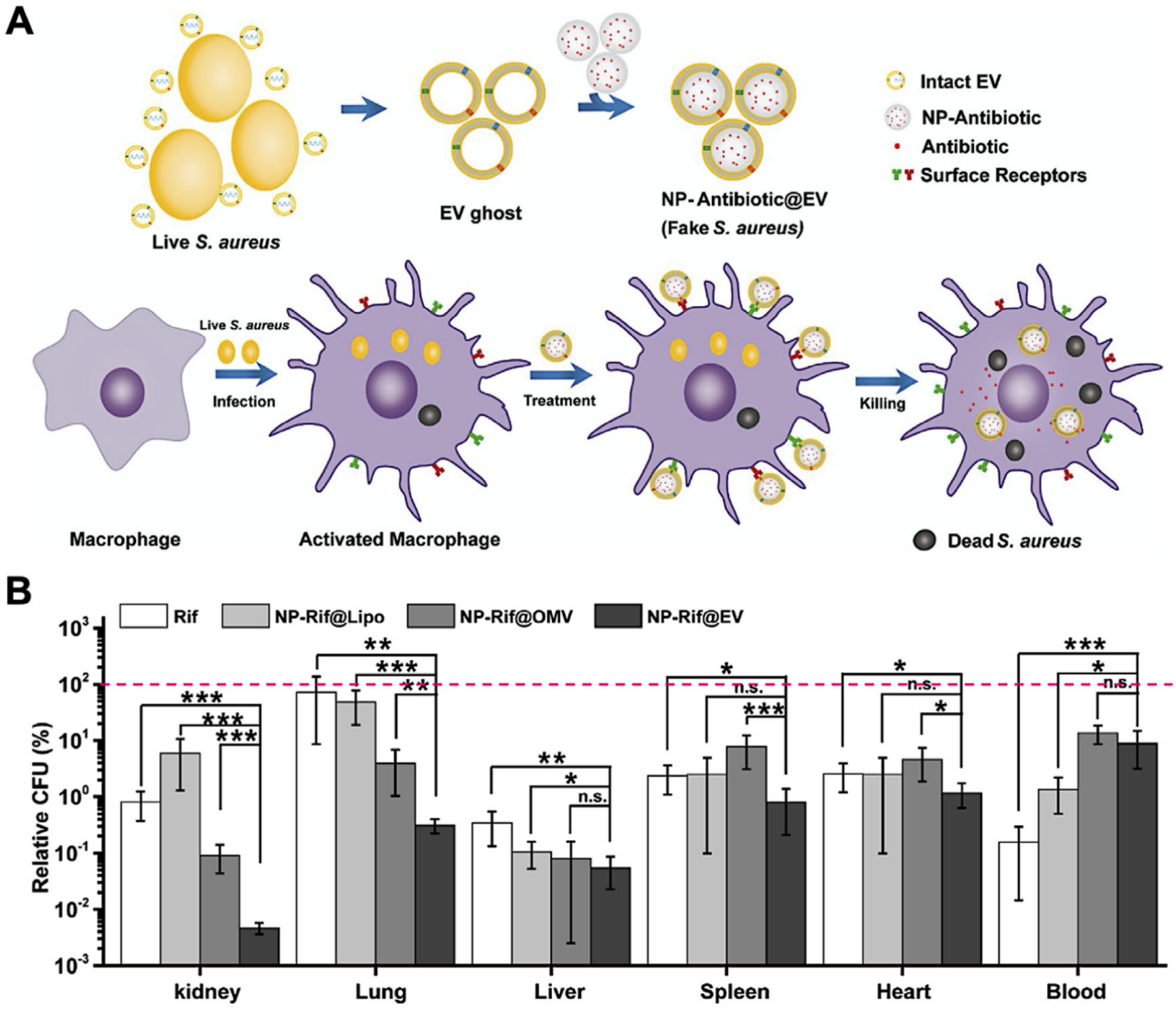


Figure 4. Bacterial membrane-coated nanoparticles (NPs) for drug delivery.

(A) Membrane-cloaked NPs were fabricated by coating *S. aureus*-derived extracellular vesicles (EVs) around antibiotic-loaded nanoparticles. Phagocytic engulfment of *S. aureus* activated macrophages and primed them for targeting by *S. aureus* EV-coated nanocarriers. (B) NPs coated with *S. aureus* EVs and loaded with the antibiotic rifampicin (Rif) significantly reduced the bacterial burden in the major organs of mice infected with *S. aureus* compared with formulations coated with a PEGylated lipid bilayer (Lipo) or *E. coli* OMVs. Adapted with permission.[74] Copyright 2018 American Chemical Society.

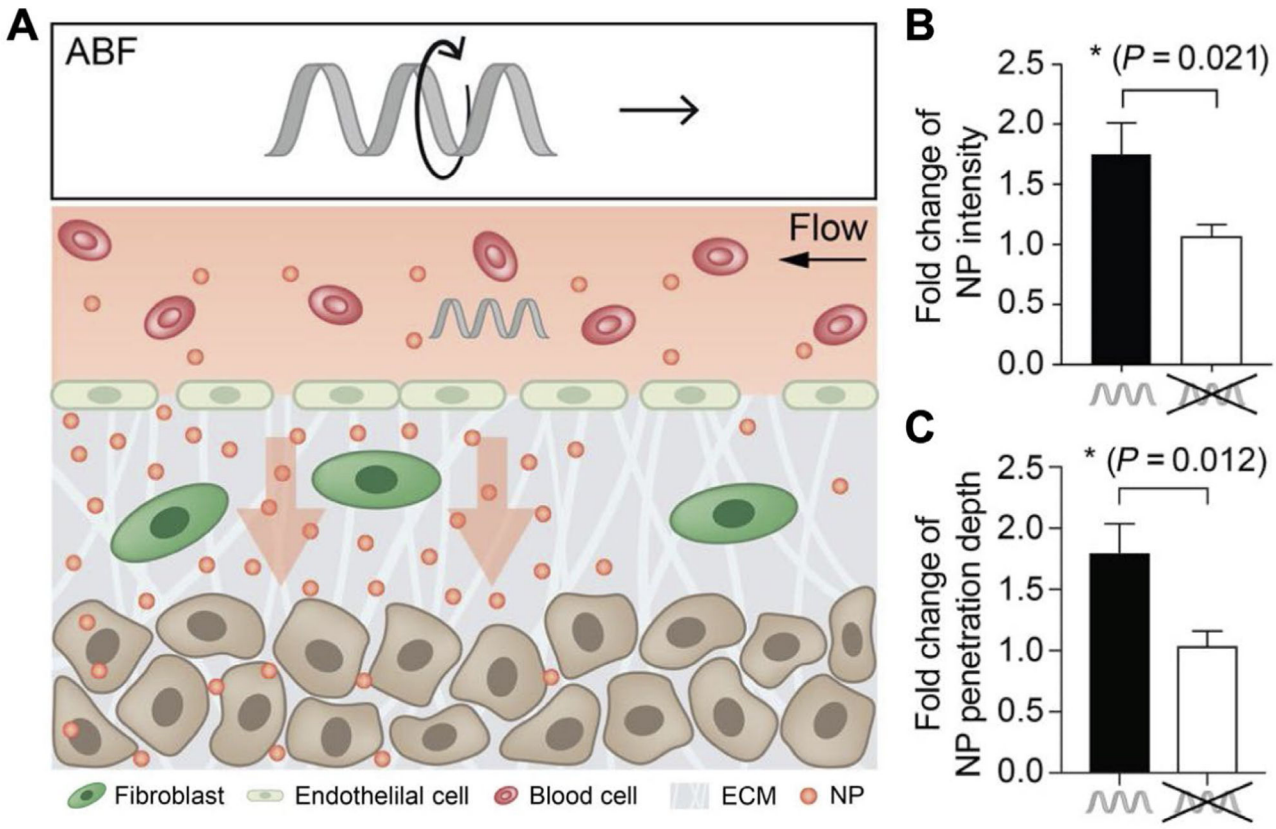


Figure 5. Bacteria-inspired synthetic microswimmers.

(A) Schematic depicting the proposed mechanism by which magnetically actuated artificial bacteria flagella (ABF) created a convective fluid flow to facilitate nanoparticle transport across endothelial barriers and into tumor tissue. (B,C) Magnetically rotating ABF enhanced the accumulation (B) and penetration (C) of nanoparticles in a microfluidic system designed to mimic the blood vessel–tumor tissue interface. Adapted with permission.[102] Copyright 2019 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science.

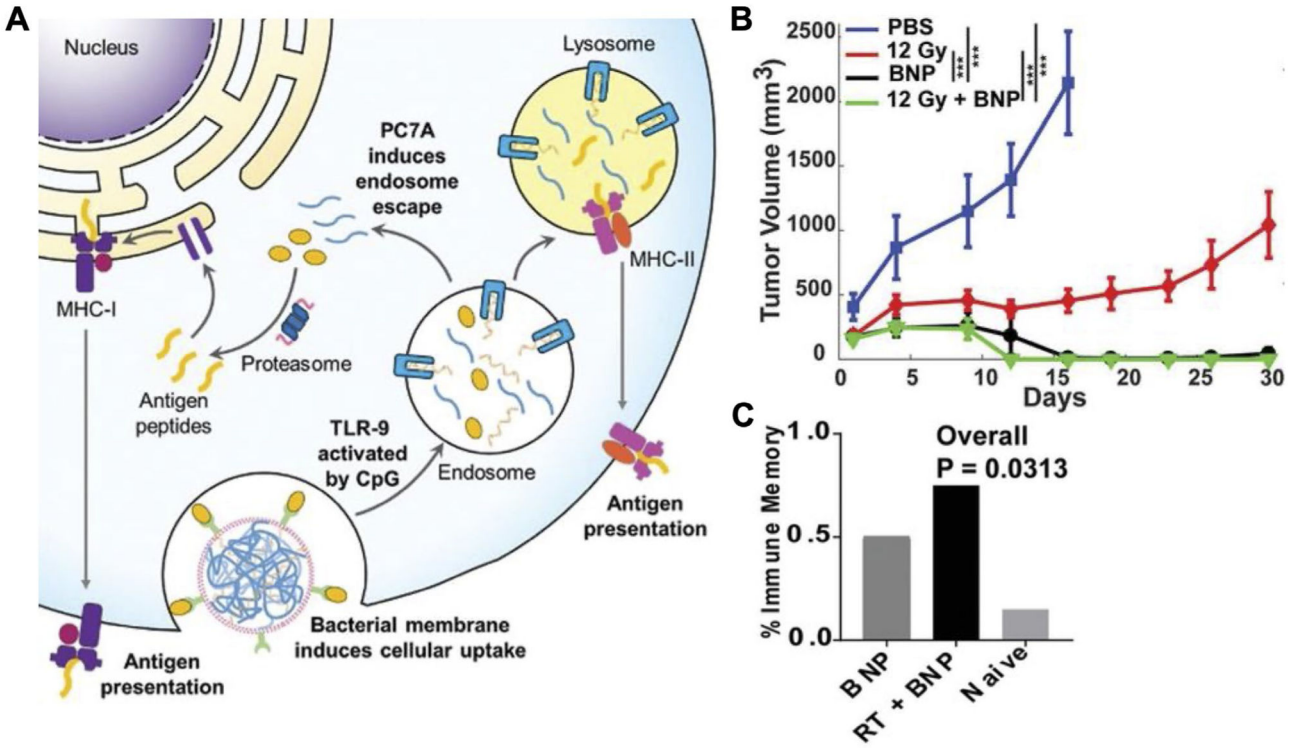


Figure 6. Bacterial membrane-coated nanoparticles (BNPs) for cancer immunotherapy.

(A) BNPs were loaded with CpG and PC7A to provide immune stimulation and endosomal escape properties, respectively, and surface-modified to efficiently capture tumor antigens after radiation therapy (RT). (B) Mice bearing NXS2 melanoma showed tumor growth reduction when treated with BNPs combined with RT. (C) When re-challenged with tumors, mice initially treated with the combination therapy exhibited more immune memory. Adapted with permission.[139] Copyright 2019 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

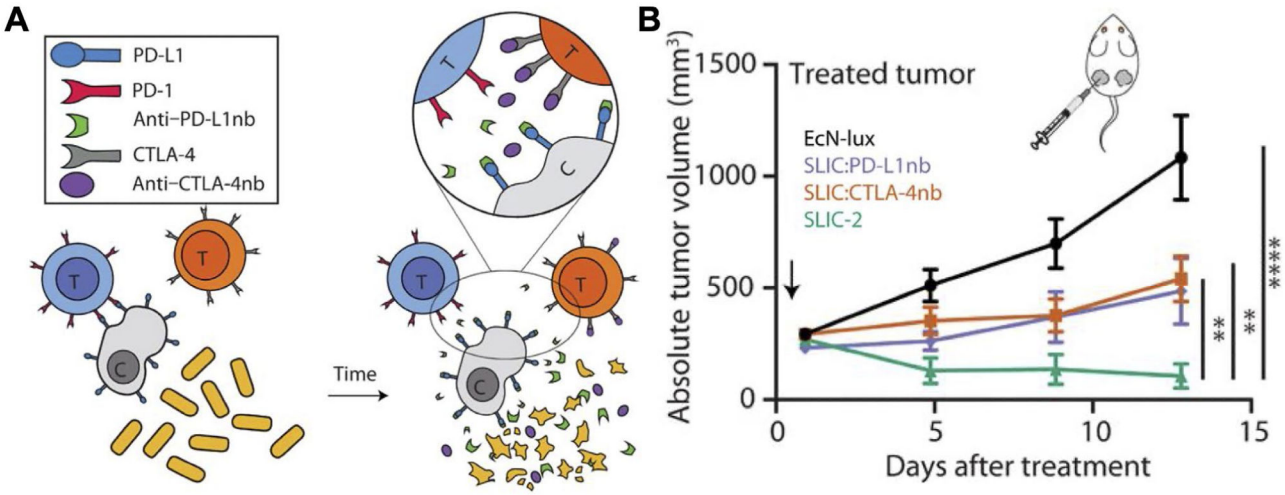


Figure 7. Bacteria-mediated nanobody delivery.

(A) Engineered bacteria allowed for controllable release of checkpoint blockade nanobodies at the tumor site, thus disrupting immunosuppressive mechanisms within the local tumor microenvironment. C: cancer cell; T: T cell; yellow rods: bacteria. (B) After intratumoral injection, bacteria with a synchronized lysis circuit (SLIC) producing nanobodies against both PD-L1 and CTLA-4 (SLIC-2) were effective in controlling tumor growth compared with control bacteria producing no nanobodies (EcN-lux), as well as bacteria producing one of the nanobodies (SLIC:PD-L1nb and SLIC:CTLA-4nb). Adapted with permission.[141]

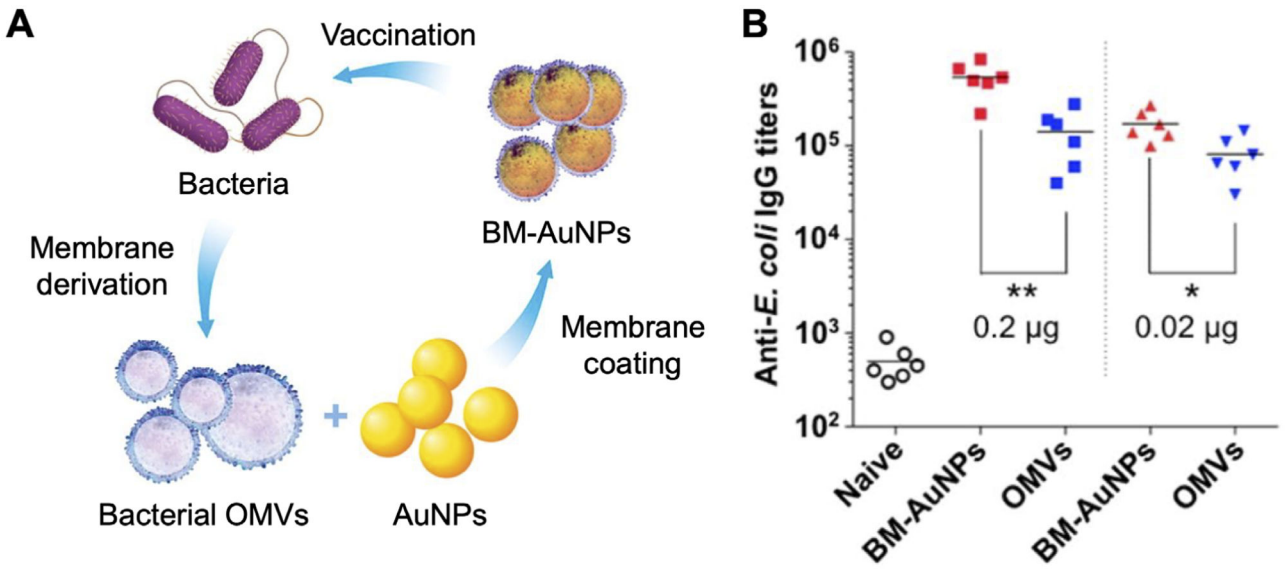


Figure 8. Bacterial membrane-coated gold nanoparticles (BM-AuNPs) for antibacterial vaccination.

(A) BM-AuNPs were fabricated by coating gold nanoparticles with *E. coli* OMVs, and the resulting formulation was used to vaccinate against the source bacteria. (B) Compared with OMVs alone, vaccination with BM-AuNPs elicited higher anti-*E. coli* immune responses. Adapted with permission.[71] Copyright 2015 American Chemical Society.

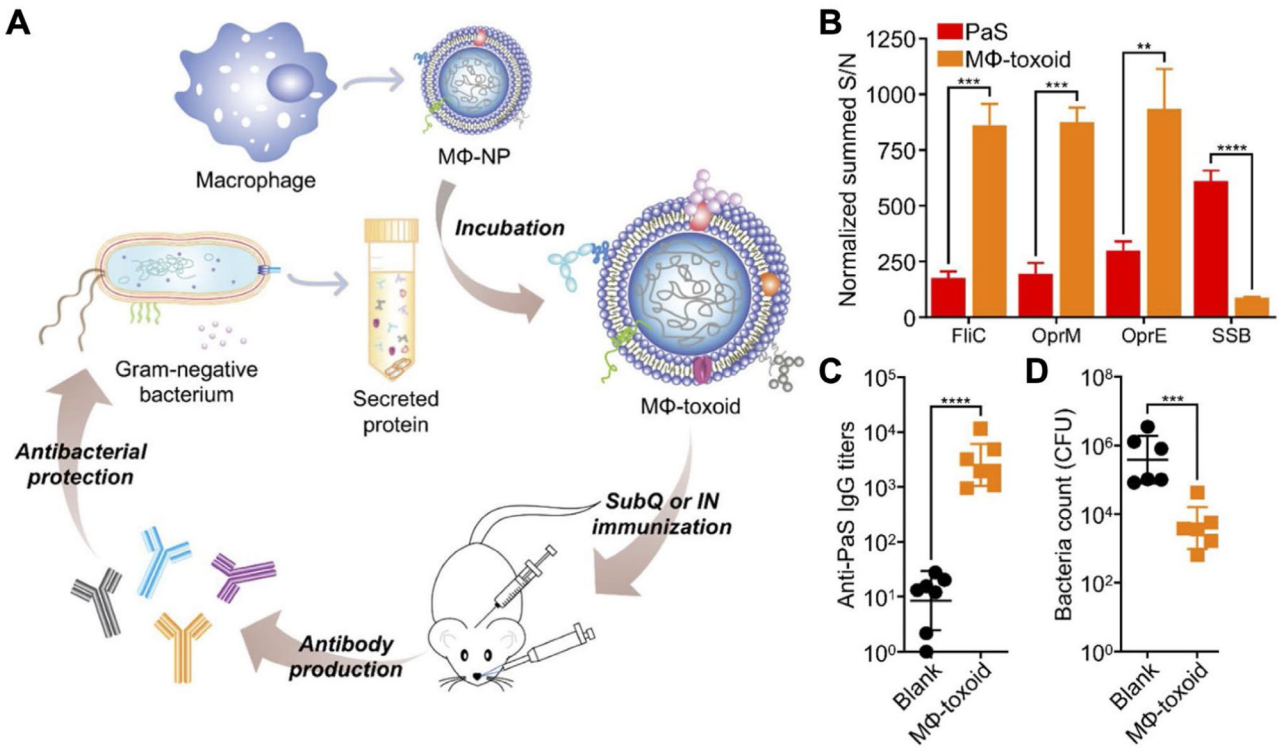


Figure 9. Bacterial protein-loaded macrophage membrane-coated nanoparticles (MΦ-NPs) as a multivalent toxoid vaccine.

(A) A MΦ-NP-based toxoid (MΦ-toxoid) was fabricated by incubating MΦ-NPs with proteins secreted from Gram-negative bacteria, and the resulting formulation was used to elicit multi-antigenic immunity. (B) Proteomic analysis demonstrated the selective enrichment of various *P. aeruginosa* secretions (PaS) on the MΦ-toxoids. (C,D) Vaccination of mice with the MΦ-toxoids resulted in higher anti-PaS titers (C) and lessened bacteria burden (D) upon intranasal challenge with live *P. aeruginosa*. Adapted with permission.[189] Copyright 2019 American Chemical Society.