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REVIEW

Progress and challenges of functionalized bacterial encapsulation: A novel biotechnology for next-generation biotherapeutics

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Targeted delivery

Abstract The disturbance of the human microbiota influences the occurrence and progression of many diseases. Live therapeutic bacteria, with their genetic manipulability, anaerobic tendencies, and immunomodulatory properties, are emerging as promising therapeutic agents. However, their clinical applications face challenges in maintaining activity and achieving precise spatiotemporal release, particularly in the harsh gastrointestinal environment. This review highlights the innovative bacterial functionalized encapsulation strategies developed through advances in physicochemical and biological techniques. We comprehensively review how bacterial encapsulation strategies can be used to provide physical barriers and enhanced adhesion properties to live microorganisms, while introducing superior material properties to live bacteria. In addition, this review outlines how bacterial surface coating can facilitate targeted delivery and precise spatiotemporal release of live bacteria. Furthermore, it elucidates their potential applications for treating different diseases, along with critical perspectives on challenges in clinical translation.

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This review comprehensively analyzes the connection between functionalized bacterial encapsulation and innovative biomedical applications, providing a theoretical reference for the development of next-generation bacterial therapies.

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1. Introduction

The gut microbiome plays a pivotal role in the onset and progression of various diseases, with its dysbiosis being closely associated with immune disorders, metabolic syndromes, and even neurological conditions[1-8]. Recent advancements highlight live therapeutic bacteria (LTBs) as a frontier in gut microbiome remodeling and next-generation biotherapeutics, leveraging their genetic manipulability, anaerobic metabolism, and immunomodulatory properties[9-11]. These microbial platforms exhibit dual therapeutic advantages: (1) They inhibit the colonization of pathogenic bacteria by secreting antimicrobial peptides (such as bacteriocins) and metabolizing short-chain fatty acids[12-14], while regulating the expression of immune factors such as interferon (IFN), interleukin (IL), and tumor necrosis factor (TNF), directly exerting the therapeutic effect of living drugs[15-18]. (2) The anaerobic tendency and metabolic plasticity of LTBs make them an ideal carrier for precise drug delivery. By attaching drugs to the bacterial surface, the enrichment efficiency of drugs at the lesion site can be significantly improved, achieving targeted sustained release while reducing systemic toxicity[19-21]. This delivery system, leveraging microbial physiological characteristics, offers a new approach for precisely treating tumors and chronic inflammatory diseases. As biocompatible therapeutic platforms, LTBs have shown strong clinical translation potential[22]. Currently, numerous clinical trials are focusing on LTBs, including fecal microbiota transplantation (FMT) and engineered bacteria therapy. However, further optimization of the delivery system and efficacy evaluation is needed. These trials include treatment of various gastrointestinal diseases[19,23-26], cancers[27,28], and even neurological diseases[29]. Puurunen et al.[30] inserted genes encoding phenylalanine ammonia-lyase and L-amino acid deaminase into the genome of *Escherichia coli* Nissle 1917, designing an engineered strain named SYNB1618, which can consume phenylalanine (Phe) in the gastrointestinal tract. The results demonstrated that SYNB1618 could significantly alleviate phenylketonuria in patients and has completed a phase 1/2a clinical trial. However, some engineered bacteria fail to achieve the results obtained in animal experiments when tested in humans. For example, the facultative anaerobe *Salmonella Typhimurium* VNP2000, which had been engineered for safety and shown anti-tumor capabilities in preclinical studies, failed in a phase I clinical trial due to its lack of significant therapeutic effects and dose-dependent side effects[31,32].

The clinical application of LTBs is currently facing two key bottlenecks: First, the harsh physiological conditions of the gastrointestinal tract, such as low pH, enzymatic degradation, and bile salts, pose significant challenges to the bioavailability and colonization ability of live bacteria[33]. Second, it is necessary to achieve spatiotemporal controlled release of live bacteria in the complex *in vivo* environment to avoid excessive proliferation, translocation, or systemic immune responses. To address these limitations, traditional microencapsulation technologies (primarily comprising emulsification, extrusion, spray drying, freeze drying, and electrospinning/electrospray) provide barrier protection for live bacteria through physical coating, significantly improving their survival rate in the gastrointestinal tract[34-36]. Nevertheless, conventional static encapsulation strategies exhibit limited capability in dynamically modulating bacterial release kinetics, particularly in complex pathophysiological microenvironments such as the tumor niche, where spatiotemporal precision is paramount. In the past decades, breakthroughs in functionalized bacterial encapsulation have integrated covalent surface modifications (polydopamine coatings, nanoparticle conjugation) with synthetic biology to create stimulus-responsive "smart" systems.

On the one hand, functional materials (such as polydopamine, liposomes, nanoparticles, and novel biomaterials) can be introduced on the bacterial surface through covalent modification or self-assembly techniques[37-41]. Bacterial surface coatings provide physical barriers[29] and introduce exogenous functionalities, such as evading immune clearance[42,43], enhancing gut adhesion[44], and loading with therapeutic components[28]. On the other hand, combining synthetic biology with bacterial surface engineering, the "triggered" release system regulated by genetic circuits can specifically respond to the hypoxia or inflammatory microenvironment of tumors, achieving site-specific release of therapeutic payloads, thereby reducing off-target toxicity and enhancing therapeutic efficacy. Integrating synthetic biology with bacterial surface engineering offers a promising approach for personalized and precision medicine. This method can customize different bacterial encapsulation strategies for different diseases (such as intestinal inflammation, tumors or infections), providing a promising approach for personalized and precision medicine[45].

As shown in Fig. 1, this review systematically examines (1) innovations in bacterial functionalized encapsulation strategies enabled by physicochemical and biological advances, (2) targeted delivery and precise spatiotemporal release mechanisms *via* oral or injectable administration, and (3) the therapeutic potential of intelligent bacterial coatings in disease intervention. The evolving understanding of gut microbiome functions is driving revolutionary bacterial-based therapies toward more precise treatment paradigms.

2. Encapsulation strategies of bacterial functionalization

Researchers have recently focused on developing functionalized bacterial encapsulation utilizing various physical, chemical, and biological approaches[46]. The most commonly used strategies include: 1) encapsulating bacteria within biocompatible polymers, lipids, or biological membranes, and 2) chemically conjugating functional molecules onto the bacterial surface. This section summarizes the common strategies and applications for the functionalized encapsulation of live bacteria as therapeutics.

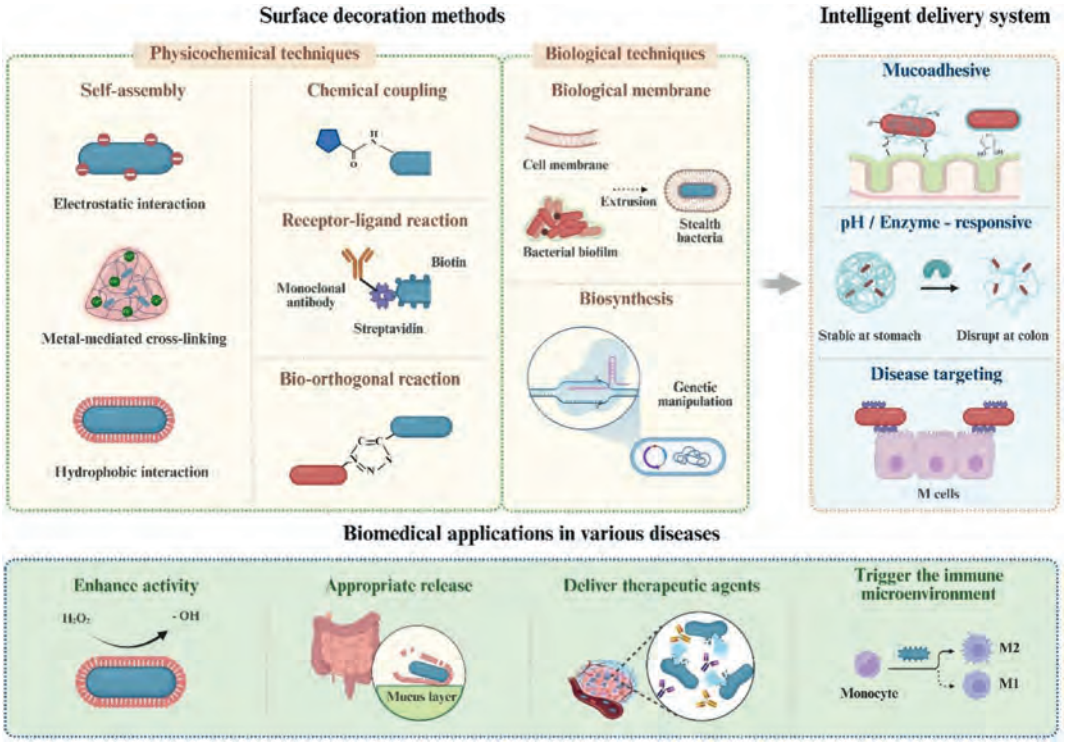

Figure 1 Schematic illustration of synergistic integration of bacterial functionalized encapsulation and biotherapeutics. The synergistic integration of bacterial functionalized encapsulation and biotherapeutics include the decoration strategies for encapsulating live bacteria, the live bacteria intelligent delivery system, and the role of functionalized encapsulation of bacteria in biomedical applications.

2.1. Self-assembly

Self-assembly is a naturally occurring phenomenon observed in various systems such as nucleic acids[47] and biofilms[48]. It involves the spontaneous organization of elementary units, such as molecules, nanomaterials, or micro/macro-scale entities, into stable and ordered structures driven by weak interactions like van der Waals forces, hydrogen bonding, and hydrophobic effects[37,38]. Inspired by natural self-assembly phenomena, researchers adapted this principle to encapsulate live bacteria. In this section, we summarize the common self-assembly strategies for live bacteria encapsulation, which are categorized based on the polymer-bacteria interactions as layer-by-layer (LBL) self-assembly and self-assembly driven by metal ions or polymer amphiphilicity (Fig. 2A).

2.1.1. Layer-by-layer self-assembly technology

LBL is a widely used strategy for live bacterial encapsulation utilizing weak interactions between molecules[49], such as electrostatic interactions, hydrogen bonding, and ligand binding[37,38]. Bacteria naturally carry various chemical functional groups on their surface, including carboxyl, hydroxyl, and phosphate groups, which give them a negative charge. Additionally, bacteria secrete negatively charged metabolites that facilitate adsorption of cationic polymers such as chitosan, polyethyleneimine, and poly (allylamine hydrochloride)[49]. Based on the electrostatic attraction between oppositely charged polyelectrolytes, the alternating adsorption of cationic and anionic polyelectrolytes on the bacterial surface leads to charge reversal, enabling layer-by-layer assembly of a multilayer membrane[49] (Fig. 2B). Tuning types, concentrations, and reaction times of polyelectrolytes allow modulation of the coating composition, thickness, and charge for improved uniformity and stability. LBL self-assembly offers simplicity and biocompatibility, and the multilayered network provides comprehensive live bacteria protection, improves live delivery capabilities, and enables colon-targeted delivery[50,51]. However, electrostatic coatings have limited stability due to the potential disruption of uniform electrostatic bacteria-polyelectrolyte interactions caused by ionic strength or pH[52].

2.1.2. Metal ion-mediated self-assembly

Oxidizing phenolic compounds using metal ions (such as Fe^{3+}, Cu^{2+}, and Al^{3+}) has been widely used for bacterial microencapsulation[39]. Tannins and polydopamine, like other materials that mimic natural tissue properties, exhibit superior adhesion and prolonged residence time in the gastrointestinal tract[39,53]. Firstly, catechol groups in phenolic compounds are oxidized to highly reactive o-benzoquinone structures under appropriate oxidative conditions. These *o*-benzoquinone intermediates are very unstable and prone to undergo a series of cross-coupling reactions and polymer assembly. Researchers[54] utilized the live bacterial essential nutrients (manganese) to induce oxidative polymerization of phenolic compounds, which simultaneously assembled to form a nano-coating around the individual strains (Fig. 2C). This bacterial encapsulation strategy demonstrates excellent biocompatibility, biodegradability, and high antioxidant activity. Additionally, with the presence of primary amine groups and thiol groups in mucins, catechol groups can react with primary amine groups and thiol groups *via* Schiff base or Michael addition to form irreversible covalent bonds, which can effectively enhance affinity with mucins in mucus. Metal ions are ubiquitous in bacterial metabolism, being essential for protein structural stability

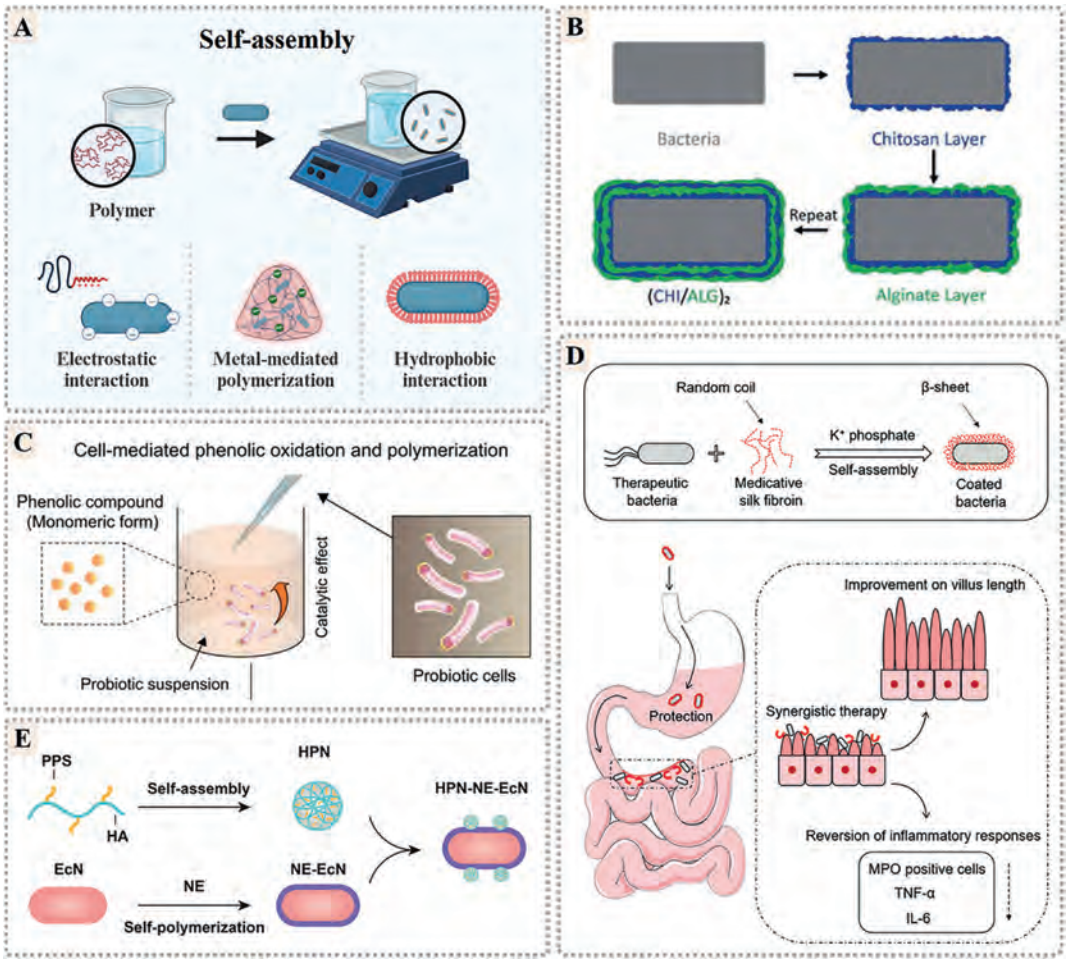

Figure 2 Self-assembly is applied to live bacteria encapsulation. (A) Schematic illustration of self-assembly *via* electrostatic interaction, metal-mediated polymerization, and hydrophobic interaction. (B) Schematic LbL templating of chitosan and alginate on live bacteria. Reprinted with the permission from Ref. 49. Copyright © 2016, John Wiley and Sons. (C) Schematic of cell-mediated oxidation of catechol compounds and its *in situ* nanoencapsulation. Reprinted with the permission from Ref. 54. Copyright © 2022, John Wiley and Sons. (D) Schematic illustration of coating therapeutic bacteria with medicative silk fibroin by biointerfacial self-assembly for synergistically enhanced biotherapy. Reprinted with the permission from Ref. 52. Copyright © 2021, John Wiley and Sons. (E) Schematic of hyaluronic acid−poly(propylene sulfide) nanoparticle (HPN) conjugation to the surface of norepinephrine (NE)-coated *Escherichia coli* Nissle 1917 (EcN). Reprinted with the permission from Ref. 57. Copyright © 2022, The American Association for the Advancement of Science.

and catalyzing various biological processes, while also being responsive to oxidative stress. Metal ion crosslinking is another strategy based on the electrostatic interactions between oppositely charged species, enabling polymer encapsulation onto bacterial surfaces. It exploits polymers with electron-donating ligand groups, such as carboxyl-containing polyacrylic acid. By introducing suitable metal ions (*e.g.*, K^+, Ca^{2+}, Zn^{2+}, Fe^{3+}) [39,52], coordination sharing ensues between the metal ions and electron-donating atoms (*e.g.*, N, O) located on the polymer chains, ultimately, leading to cross-linking. This crosslinking induces alterations in polymer conformation and enables self-assembly into three-dimensional hydrogel networks at the bio-interface, which effectively embeds and protects bacteria[52] (Fig. 2D). In all, this self-assembly strategy is operationally simple and mild, minimally affecting bacterial viability[39].

Although the self-assembly strategy based on metal ion cross-linking is one of the classical strategies for bacterial encapsulation, the network structure is not as stable and homogeneous as covalent cross-linking, and polymer degradation may occur after prolonged use, leading to the loss of encapsulation. Furthermore, some metal ions may be toxic to bacteria, and their cross-linking ability can be compromised by interfering ions. Therefore, selection and operation conditions of metal ion species are crucial in live bacterial encapsulation to minimize the adverse effects.

2.1.3. *Self-assembly based on hydrophobic interactions*

Amphiphilic polymers have the ability to undergo microphase separation and self-assemble into various well-defined assemblies with ordered microstructures ranging from nanometers to micrometers in size, such as micelles, vesicles, and nanoparticles. Taking advantage of the lipophilic properties of bacterial membranes[55,56], the self-assembly of amphiphilic polymers on bacterial surfaces has emerged as a new strategy for functionalized bacterial encapsulation[57].

The self-assembly of amphiphilic polymers is mainly determined by their intrinsic properties in solution. Firstly, some polymers naturally possess amphiphilicity. For example, cereal-derived prolamins rich in proline and glutamine residues can

easily self-assemble into micro/nanoparticles[58,59]. Secondly, the conjugation of polymers with opposing affinities can yield amphiphilic block copolymers. As shown in Fig. 2E, Liu et al.[57] synthesized a lipophilic molecule, poly(propylene sulfide), that scavenged reactive oxygen species (ROS), which was then coupled with hyaluronic acid (HA) to form an HA−PPS amphiphilic block copolymer. The hydrophobic block facilitated random aggregation into a core surrounded by a soluble HA shell, self-assembling into nanoparticles while addressing the poor water solubility of PPS. Finally, hydrophilic polymers can be chemically modified with various hydrophobic moieties[47], such as porphyrins, cholesterol, azo-benzenes, and ethylthiophosphate esters, thereby converting them into amphiphiles. Therefore, exploiting electrostatic and hydrophobic interactions between the modified polymers and bacteria presents a promising approach for bacterial encapsulation, considering the presence of negatively charged lipid bilayers on bacterial surfaces[40].

Hydrophobic interactions, while providing an efficient driving force for self-assembly, still face multiple challenges that need to be overcome in practical applications. Firstly, the high hydrophobicity of hydrophobic groups may lead to biocompatibility issues. Excessive hydrophobicity can cause non-specific fusion with cell membranes, triggering cytotoxicity[60,61]. This toxic effect is particularly significant in cationic assemblies, thus requiring precise balancing of the ratio of hydrophobic to charged groups through molecular design. Secondly, hydrophobic interactions themselves lack directionality and rely on other forces (such as hydrogen bonds and $\pi-\pi$ stacking) to guide ordered assembly[62]. Hydrophobic interactions are susceptible to factors such as solvent polarity and temperature. The complex *in vivo* environment (*e.g.*, proteases, ionic strength) may shorten the structure of hydrophobic self-assemblies[63]. Future research combining molecular dynamics simulations and machine learning could predict the mechanisms of polymer-membrane interactions to optimize the hydrophobic/hydrophilic balance, achieving more precise functional self-assembly systems.

2.2. *Chemical modification of bacterial surface components*

The peptidoglycans, lipopolysaccharides, lipids, and proteins on the bacterial cell membranes are crucial for their interaction with the surrounding environment, which also offers opportunities for the chemical conjugation of functional materials to the bacterial surface[40]. Single-cell surface chemical modification techniques targeting bacterial components represent an emerging interdisciplinary approach for precise drug delivery[46,53,64]. Common bacterial surface modifications involve either *in vitro* chemical conjugation or *in vivo* labeling of their metabolites, allowing the introduction of exogenous functionalities that are unattainable by natural means as depicted in Fig. 3A. The chemical modification of bacterial surface components is to achieve controlled release and enhance therapeutic bioavailability, which leverages the unique structural properties and metabolic activities of bacteria and integrates synthetic biology, nanotechnology, and materials science. Therefore, intelligent therapy using live bacteria as carriers is a cutting-edge field applicable to the treatment of a wide range of diseases[19,53,64-66].

2.2.1. *Chemical coupling*

The bacterial surface consists of a series of distinct compositions, such as membrane, cell wall, capsules, and flagella, all of which share conserved structural domains[67] and provide a universal foundation for conjugating artificial functional materials[68,69]. Recently, Lin et al.[46] comprehensively reviewed chemical modification methods based on different bacterial surface structures, serving as an essential reference for realizing unique functionalities. This section focuses on common covalent modification approaches, such as amide formation *via* amino/carboxyl groups and Michael addition reactions of thiols[64]. These approaches enable the conjugation of exogenous functional systems onto the bacterial surface, enhancing the therapeutic capabilities of live microbial therapeutics[70].

Firstly, the integration of exogenous functional modules serves not only to enhance the tolerance of live bacteria against adverse environments but also to promote their accumulation and precise release at disease sites, making it more suitable for precision medicine. For example, mimicking antioxidant defense systems can protect bacteria from ROS-induced damage[19]. Secondly, exploiting their anaerobic propensity, immunomodulatory effects, and tumor-targeting capabilities[53], live bacteria can serve as drug carriers by covalently conjugating functional materials (*e.g.*, anticancer drugs[64] or immunomodulators[53]). This provides a novel approach for constructing "biohybrid" theranostics[19] and an ideal strategy for long-term, multimodal reversal of tumor immunosuppressive microenvironments[70]. As shown in Fig. 3B, Han et al.[64] covalently conjugated *Clostridium butyricum* spores with gemcitabine-loaded mesoporous silica nanoparticles (defined as MGEM) *via* amide formation between amino and carboxyl groups to construct an oral drug delivery system (SPORE-MGEM). This system leveraged the gut−pancreas axis to enhance chemotherapeutic effects against pancreatic ductal adenocarcinoma without significant side effects.

The chemical conjugation-based bacterial encapsulation technology has significant advantages in improving bacterial tolerance, enhancing drug delivery efficiency, and achieving multifunctionality[19,70]. However, its application also faces some challenges. Firstly, in certain cases, chemical modifications may interfere with the normal physiological functions of bacteria. For example, excessive modification can lead to the loss of bacteria's natural immune evasion mechanisms or metabolic capabilities. Secondly, chemical conjugation requires strict control of reaction conditions (such as temperature, pH, and time), otherwise it may produce toxic by-products or reduce conjugation efficiency[64]. This demands more time and resources in experimental design. Lastly, compared to physical encapsulation methods, chemical conjugation involves complex chemical reactions and higher costs. Future research can overcome these limitations by optimizing reaction conditions, developing new modifying agents, and integrating other technical means, thereby further enhancing the application potential of this technology.

2.2.2. *Receptor−ligand binding*

Receptor−ligand interactions orchestrate various biological processes through intercellular communication[71]. Among them, antigen−antibody recognition and biotin−avidin binding are the most commonly exploited forms of specific receptor−ligand complexation[41,71]. Recently, antibody−drug conjugates (ADCs) have gained attention as a targeted drug delivery modality, as they can directly deliver potent anticancer drugs to targeted cells by conjugating to the tumor-specific antibodies, maximizing therapeutic efficacy while minimizing damage to the healthy cells[72]. The core of ADCs technology lies in optimizing the conjugation chemistry and innovating the drug carriers. For instance, Li et al.[53] developed the bacterial decoration with triple immune nano

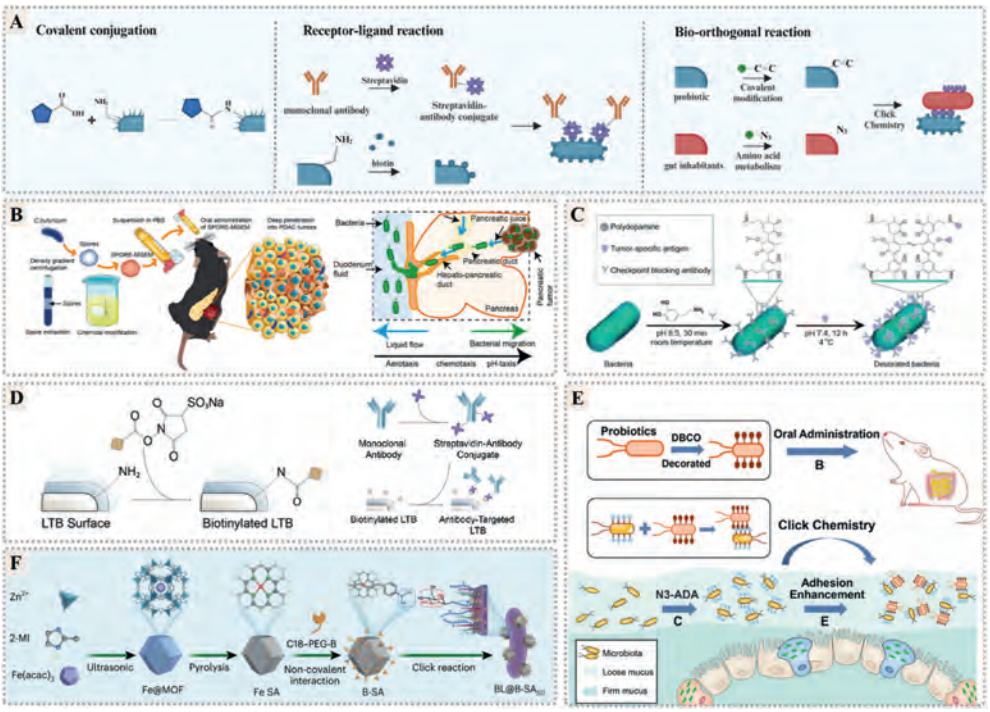

Figure 3 Chemical modification of bacterial surface components. (A) Common bacterial surface modifications can be divided into chemical coupling, receptor and ligand binding, and *in vivo* labeling based on the bacterial own metabolism, thereby introducing exogenous functions that cannot be achieved naturally. (B) Construction of *Clostridium butyricum* spore-based oral drug delivery system for improving pancreatic ductal adenocarcinoma targeting and enhancing chemotherapy efficacy. Reprinted with the permission from Ref. 64. Copyright © 2022, American Chemical Society. (C) Schematic illustration of preparing tumor-resident living immunotherapeutics by decorating bacteria with triple immune nanoactivators. Reprinted with the permission from Ref. 53. Copyright © 2022, John Wiley and Sons. (D) *N*-Hydroxysulfosuccinimide ester chemistry for bioconjugation of biotin to primary amines on the bacteria surface. Streptavidin conjugation to the constant region of IgG antibodies enables antibody attachment to the surface of biotinylated bacteria. Reprinted with the permission from Ref. 41. Copyright © 2020, John Wiley and Sons. (E) Schematic illustration of bioorthogonal mediated bacterial delivery to enhance probiotics colonization in the gut. Reprinted with the permission from Ref. 44. Copyright © 2022, The Authors. Published by American Chemical Society. (F) Schematic illustration of Redox-regulated nanozymes were linked to a range of clinically relevant probiotics *via* click chemistry to build a probiotics@B-FeAu library for adaptive cancer therapy. Reprinted with the permission from Ref. 28. Copyright © 2023, John Wiley and Sons.

activators as tumor-resident living immunotherapeutics, in which tumor-specific antigens and checkpoint blocking antibodies are simultaneously conjugated onto the bacterial surface under cyto-compatible conditions. Then, polydopamine (PDA) nanoparticles are formed *via in situ* dopamine polymerization. The decorated bacteria showed spatiotemporal tumor retention and proliferation-dependent drug release, displaying great potential for treating colon cancer (Fig. 3C).

Biotin, ubiquitous in plant and animal tissues, possesses two cyclic structures: an imidazolone ring and a thiophene ring. The imidazolone ring constitutes the primary avidin-binding site, while the terminal carboxyl group of the thiophene ring serves as the sole moiety for antibody/biomolecule conjugation. Avidin (AV), extracted from egg albumin, is a basic glycoprotein comprising four identical subunits[73], exhibiting thermal stability and resistance to various proteolytic enzymes, particularly when bound to biotin. Streptavidin (SA), secreted by *Streptomyces avidinii*, comprises four identical nonglycosylated peptide chains, enhancing its versatility over AV. Owing to their high-affinity binding specificity and biosafety, biotin and SA are extensively utilized as oral supplements, particularly in targeted cancer therapy[65,66]. Inspired by the bacterial adhesins that facilitate colonization, Vargason et al.[41] developed synthetic adhesins for LTBs. They used *N*-hydroxysuccinimide (NHS) chemistry to form amide bonds between the ubiquitous surface amines on bacteria and the carboxyl groups of biotin, thereby conjugating biotin onto the bacterial surface. This improves bacterial colonization within the gastrointestinal (GI) tract and can be applied to any synthetic adhesin target or LTBs species (Fig. 3D).

The bacterial encapsulation strategy based on receptor−ligand interactions demonstrates unique advantages in the field of synthetic biology therapy through specific molecular recognition mechanisms. These advantages include high specificity, enhanced drug delivery effects, multifunctional design, and improved bacterial adhesion capabilities[53]. However, issues such as non-specific binding may arise during biotin−avidin interactions, and the binding process can be interfered with by background signals. Additionally, the ligand immobilization process involves cumbersome surface engineering modifications, including optimization of transmembrane protein anchoring domains and insertion of non-natural amino acids, which are key technical aspects. The stability and batch-to-batch consistency of these processes still need improvement[41]. Therefore, future research should focus on optimizing coupling chemistry, reducing non-specific signals, and developing more cost-effective technical solutions to further enhance their feasibility and efficiency in practical applications.

2.2.3. Bioorthogonal reactions

Natural products often form carbon−heteroatom bonds to create complex molecular structures, allowing for the efficient synthesis of diverse biomolecules such as proteins and polysaccharides[74-77]. Click chemistry reactions exhibit exquisite reaction specificity and substantial thermodynamic driving forces, facilitating rapid proceedings even in complex physiological environments. Inspired by the natural synthesis, Bertozzi's gourp[76,77] developed bioorthogonal reactions to modify the important but elusive biomolecules like polysaccharides and carbohydrates on bacterial surfaces. These bioorthogonal reactions can occur under complex physiological conditions without interfering with other biological processes or damaging the living system and target biomolecules. Currently, utilizing bioorthogonal chemistry to control the timing and location of drug release is a highly promising application, as it can generate drugs with selective cytotoxicity towards target cells and improve the outcome of targeted cancer therapies[78].

In the field of bacterial surface modification, bioorthogonal reactions provide a convenient and efficient approach for conjugating exogenous functional materials to bacterial cell walls. Cao et al.[19] coupled the phenylboronic acid functional groups derived from the pH-sensitive peroxidase-like (POD-like) nanozyme to the polysaccharides of the cell wall of *Bifidobacterium longum via* click chemistry, which enhanced antitumor therapeutic responses. By introducing exogenous chemical functional groups directly onto the bacterial surface through amino acid metabolic engineering with click chemistry, reactive sites for subsequent chemical reactions can be created, offering a novel strategy for delivering therapeutic payloads *via* live bacteria. For instance, Song et al.[44] developed a bioorthogonal-mediated delivery strategy by metabolically incorporating azide-modified alanine into gut inhabitants' peptidoglycans for azido decoration, and then conjugating dibenzocyclooctyne (DBCO) to live bacteria through amide bonds. Upon oral administration, the DBCO-modified bacteria bioorthogonally bound to azide-modified gut inhabitants, enhancing their colonization through bacterial adhesion in the complex physiological environments (Fig. 3E). This bioorthogonal strategy utilizes metabolically labeled gut commensals as artificial reactive sites for *in situ* conjugation, providing a promising approach to improve bacterial delivery and implantation.

Encapsulation strategies based on bioorthogonal chemistry offer significant advantages in enhancing the efficiency of bacterial surface functionalization, achieving precise drug delivery, and augmenting therapeutic efficacy[28,79] (Fig. 3F). However, this technology faces notable limitations. Firstly, the metabolic labeling process may alter the surface charge distribution of bacterial membranes, thereby affecting their ability to colonize the gut. Bioorthogonal reactions typically require conditions close to physiological ones (such as room temperature and neutral pH), which precludes many chemical reactions that necessitate high temperatures or strong acidic or alkaline conditions[80,81].

2.3. Biological techniques

2.3.1. Membranes of biological origin

The delivery of overdose bacteria, especially engineered bacteria, may trigger inflammatory responses in the host and accelerate their clearance[21]. One approach to reduce the immunogenicity and toxicity of LTBs is through synthetic biology, by genetically eliminating bacterial surface antigens such as lipopolysaccharides[82]. However, this strategy may lead to a decrease in activity and alteration of strain function. Another approach is to coat microorganisms to avoid immune recognition[42]. Recently, biologically derived membranes, known for their high biocompatibility, stability, and low immunogenicity, have been utilized for bacterial encapsulation, balancing the efficacy and safety of LTBs. Furthermore, they can enhance mucosal adhesion in the target area and promote therapeutic effects. Currently, the biologically derived membranes for bacterial encapsulation are primarily derived from bacterial biofilms, cell membranes, and their derivatives through membrane extraction and biomimetic synthesis (Fig. 4A).

In nature, biofilms protect bacteria from environmental stressors like gastric acid and antibiotics[83]. They help the bacteria occupy a dominant ecological niche in the mucosa, preventing pathogen invasion[84]. Recent research has proved that the Toll-like receptors of the immune system can distinguish between the extracellular matrix produced by pathogens and probiotics[85,86]. Therefore, inducing commensal gut bacteria to secrete biofilms or their components as encapsulants, such as extracellular polysaccharides, can help exogenous bacteria escape host immune clearance, thereby achieving the mission of targeted colonization[87,88]. Inspired by their natural characteristics, Wang et al.[83] designed a bioinspired strategy of self-coating with biofilms, which endowed the transplanted gut microbiota with superior resistance and adhesion capacity (Fig. 4B).

Additionally, live bacteria can be encapsulated within reconstituted cell membranes derived from human blood cells or tumor cell-derived apoptotic bodies using physical methods such as extrusion and sonication[42]. This encapsulation strategy has several outstanding advantages. Firstly, it provides a protective barrier that enables live bacteria to escape immune surveillance and increase retention *in vivo*, while inhibiting the toxin release and reducing side effects. Secondly, by introducing specific targeting moieties or natural targeting properties of the cell membranes, such as tumor cell membranes, the encapsulated bacteria can be delivered more precisely to the desired sites or tissues. Furthermore, the immunomodulatory effects of cell membranes are crucial for the treatment of many diseases, which can regulate the immune response such as immune checkpoint inhibitors therapy[89,90], CAR-T cell therapy[91], and tumor vaccines therapy[42] (Fig. 4D). Recently, Liu et al.[92] encapsulated live bacteria with tumor cell-derived apoptotic vesicles. This therapy ingeniously combines the unique biological properties of tumor cells and bacteria to form an innovative method for cancer treatment. The presence of tumor antigens on the CCMB surface triggers a specific anti-tumor immune response, resulting in dual-mode specific immunity and microbial therapy (Fig. 4C).

Given that it has been demonstrated that a variety of intestinal probiotic bacteria can be induced to produce extracellular matrix under certain conditions, the above methods are universally applicable and have high biosafety, thus showing significant prospects for clinical transformation. However, the use of bio-based membrane coating strategies has limitations. Such strategies do not allow for *in situ* modulation and are disposable, which may result in unexpected bacterial overgrowth and tissue toxicity. Further exploration by researchers is still needed.

2.3.2. Synthetic bioengineering

The next frontier in microbial therapeutics involves their genetic modification[9]. Engineered live bacteria that are modified using synthetic biology strategies are more desirable to be evaluated as drugs or drug delivery vectors[94]. In the biomedical field, synthetic

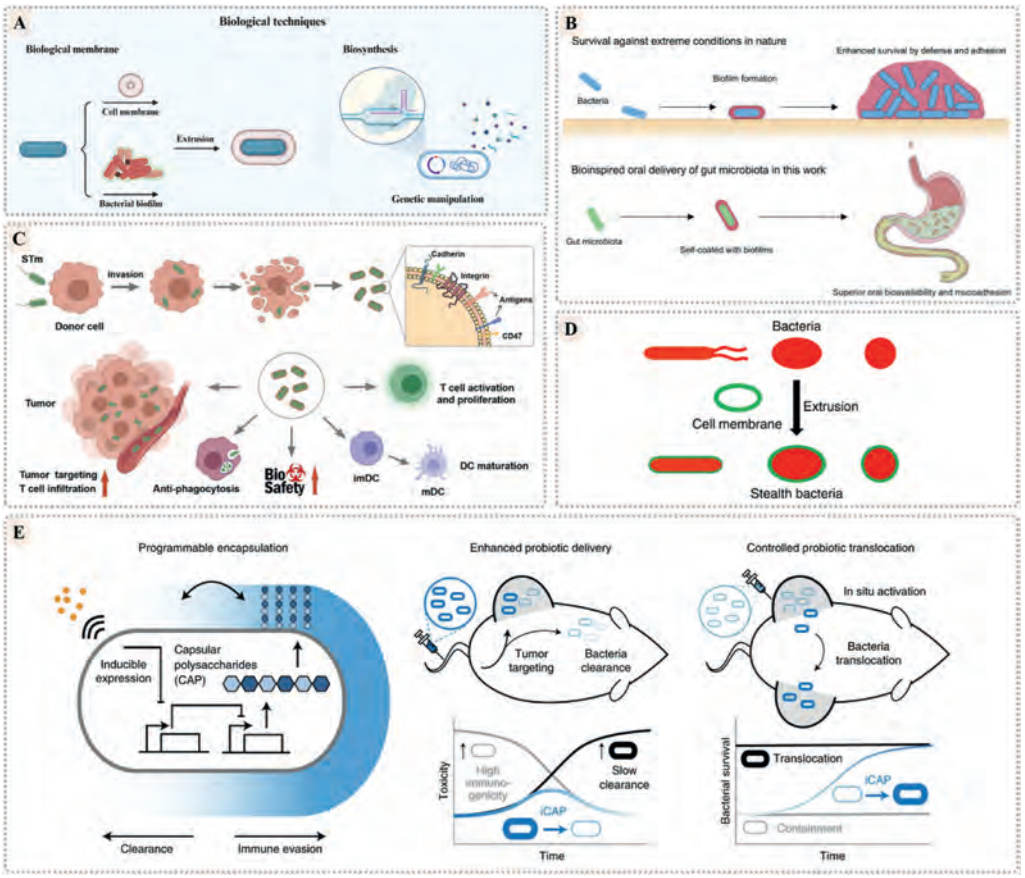

Figure 4 Surface decoration of bacteria *via* biological techniques. (A) Bioinspired oral delivery of gut microbiota with superior oral bioavailability and mucoadhesion by self-coating with biofilms. (B) Schematic illustration of biofilm formation of bacteria in nature and bioinspired oral delivery of gut microbiota with superior oral bioavailability and mucoadhesion by self-coating with biofilms. Reprinted with the permission from Ref. 83. Copyright © 2020, The American Association for the Advancement of Science. (C) Schematic illustration of the preparation of multimodal tumor-loving bacteria with tumor cell-derived nanoshell coatings and their mechanism of action. Reprinted with the permission from Ref. 92. Copyright © 2022, Elsevier. (D) Cell-membrane-coated bacteria (CMCB) is a strategy to generate stealth bacteria by camouflaging with cell membrane, prepared by simply extruding erythrocyte membranes with bacteria. Reprinted with the permission from Ref. 42. Copyright © 2019, Springer Nature. (E) Inspired by its natural ability to protect live bacteria, researchers applied a synthetic biology approach to genetically engineer CAP biosynthesis for enhanced microbial delivery *in vivo*. Reprinted with the permission from Ref. 93. Copyright © 2022, Springer Nature.

biology strategies can attenuate the virulence of pathogens and reduce their immunogenicity. They can also improve the adherence of the vector bacteria by increasing their secretion of mucoadhesive substances[93,95], or enhancing their specificity of targeting lesions[96,97]. Additionally, synthetic biology strategies can help live microorganisms cope with the harsh environment of the gastrointestinal tract and the overactive inflammation in lesions[19]. Currently, the efficiency of bioengineering bacteria has garnered widespread recognition, and the U.S. Food and Drug Administration (FDA) is actively engaged in the thorough review of the efficacy and safety profiles of several "live bacterial vectors" or "live biotherapeutic products"[10,98].

Currently, synthetic biology is widely used to generate materials from live bacteria similar to natural biomaterials, elevating the precision of oral delivery systems[99]. For instance, researchers have employed synthetic biology to improve the protective effect of engineered EcN against DSS-induced colitis in mice by enabling it to produce coiled nanofibers, CsgA, *in situ*[100]. Natural biomaterials with autonomous modes can sense and respond to the environment to achieve self-assembly[101]. Another important natural extracellular biopolymer is capsular polysaccharide (CAP), which initiates the process of bacterial biofilm formation to enhance their adhesion to biotic or abiotic interfaces[95]. Harimoto et al.[93] applied synthetic biology methods to construct a programmable surface capsular polysaccharide expression system that enhances systemic delivery, termed inducible CAP or iCAP, *via* an external inducer, IPTG. This system can control bacterial immunogenicity and survivability *in vivo*, enabling increased dosing and *in situ* trafficking to maximize therapeutic efficacy and safety (Fig. 4E). In addition to its application in cancer therapy, the surface coating strategy of programmable living microorganisms can be extended to other clinical settings. Table 1 summarizes the recent achievements in the encapsulation of bacteria and their functions achieved through different methods and materials.

Bacterial therapeutics based on synthetic biology have shown great potential in the fields of disease diagnosis, treatment, and delivery[102]. However, their long-term safety in the human body still needs to be further verified. For example, live bacterial therapies may trigger immune responses or potential toxicity[103]. In addition, engineered bacteria may spread into the environment and exchange

Table 1 Representative reports about coating materials used to encapsulate bacteria.

Modification method	Probiotic	Encapsulating material	Advanced functionality	Ref.
Self-assembly	*E. coli* Nissle 1917	Eudragit L100, Tannins (TA), Calcium ions	Increase the retention time of EcN by adhering to the mucosa, without influencing the probiotics' growth and proliferation	39
Ionic crosslinking	*L. Plantarum*	Sodium alginate, TEMPO-oxidized cellulose nanofibers	Stabilize in simulated gastric fluid, but will swell in simulated intestinal fluid	113
Extrusion/Ionic crosslink	*L. plantarum*	Pectin/Starch/Ca^{2+}	Increase the tolerance of *L. plantarum* to strongly acidic media and bile solutions and achieve targeted release	114
Layer-by-layer	*B. coagulans*	Alginate, chitosan	Against acidic and bile salt insults, mucoadhesion and growth on intestinal tissues	49
Co-deposition	*E. coli* Nissle 1917	PDA, chitosan	Show more than 30 times higher bioavailability in the gut and fourfold higher accumulation in the inflamed tissue	115
In situ nanoencapsulation	*L. rhamnosus*, *L. helveticus*, *L. plantarum*	Dopamine (DA) and plant polyphenols	Improve gastric tolerance, enhance adhesion as high and exhibit a high antioxidant activity	54
Self-assembly	*E. coli* Nissle 1917	Silk fibroin	Wrap with a therapeutic nanocoating as a unique strategy to invent diverse functional microecologics for enhanced oral delivery and therapy	52
Covalent conjugation	*C. butyricum* spores	Mesoporous silica nanoparticles loaded with the potent cytotoxic drug gemcitabine	Leverage the active targeting of natural bacteria *via* gut −pancreas axis translocation for PDAC therapy to reduce the risk of tumor metastasis	64
Covalent conjugation	*E. coli* Nissle 1917	Traut's reagent	Surface-thiolated bacteria show advantages in targeting and colonize tissues characterized with thick mucus	116
Covalent conjugation	*S. typhimurium* VNP20009	Aptamer AS1411	Enhance antitumor efficacy in tumor-bearing mouse models, accompanied with remarkably elicited immune responses within the tumor	21
Covalent conjugation	*E. coli* Nissle 1917	Dopamine, tumor-specific antigens and checkpoint blocking antibodies	Show spatiotemporal tumor retention and proliferation-dependent drug release, achieving potent antitumor effects	53
Covalent conjugation/ Receptor and ligand binding	*L. casei*, *E. coli*, and *B. coagulans*	Biotin and synthetic adhesins	Alter short-term intestinal transit and improve LTB colonization and pharmacokinetics	41
Extrusion	*E. coli* Nissle 1917	Yeast cell membrane	Initiate of mucosal immune responses and protect coated EcN from the insults of the gastrointestinal environments	117
Click reaction	*B. longum*	Peroxidase-like artificial enzymes	Reduce ROS levels, inhibit proinflammatory cytokine production, restore the intestinal barrier functions, and increase the richness and diversity of the gut microbiota	28
Bioorthogonal conjugation	*C. butyricum*	Azide and dibenzocyclooctyne	Counteract the complex intestinal environment and achieve enhanced delivery of bacteria in the GI tract	44
Biological membrane	*S. typhimurium*	Tumor cell membranes	Show improved safety and tumor localization, and a dual antigen-specific immunotherapy and microbial therapy	92
Synthetic biology	*E. coli* Nissle 1917	Capsular polysaccharide	Increase in maximum tolerated dose of bacteria and improved anti-tumor efficacy in murine models of cancer	118
3D printing technology	*A. xylinum* and *B. subtilis*	Hyaluronic acid and κ-carrageenan	Be used for bioremediation and complex-shaped synthetic skin scaffolds for biomedical applications	102
Microfluidic technology	*the genus Escherichia* and *Bacillus*	Calcium alginate and polydopamine	Enable an increase of the degradation rate of nitrogenous wastes in complex environments	119
Microfluidic technology	*L. plantarum*	Pluronic®, acrylic acid (PPP12), okara oil	Protect probiotics from the gastric environment, enabling their release in the gut	104

genes with other microorganisms, thereby posing ecological risks[104,105]. Engineered bacteria may have off-target effects under certain conditions, causing damage to non-target cells or tissues. For example, in tumor treatment, bacteria may be toxic to normal tissues. Future research needs to further optimize the design and production process of engineered bacteria, and strengthen their safety assessment and clinical translation research in humans[106].

In conclusion, surface decoration of bacteria, as an advanced biomedical approach, seamlessly integrates chemical, physical, and bioengineering methods, combining the inherent functionalities of bacteria with the advantages of additional materials. The integration of surface modification and internal genetic editing provides a new perspective for the clinical translation of live bacteria as drug carriers and biotherapeutics. In addition, novel

technologies have been applied to live bacteria encapsulation, mainly 3D printing[107] and microfluidics[108], which have made the process more miniaturized[108-110], integrated, intelligent[111,112], and biomimetic[107]. Furthermore, the integration of multiple technologies helps LTBs deliver multiple interactive contents without interfering with each other and also provides the possibility of incorporating prebiotics and other functional materials[108,109]. Looking ahead, there will be a growing trend towards the development of personalized, application-oriented bacterial encapsulated products tailored to different diseases and consumer populations, thereby revolutionizing the landscape of LTBs.

3. Live bacteria intelligent release system

Achieving precise release at the target site is another key focus in developing oral live bacteria delivery systems[120]. Considering the physiological differences in various organs, the use of appropriate biomaterial polymers and bacterial release strategies can help live bacteria overcome the hostile environment[121,122], precisely release and long-term colonization at the intended site (Fig. 5). Similarly, referring to the classification of intelligent bioactive substance delivery systems [123], oral live bacteria delivery systems can be categorized into four types: 1) Mucus-adhesive systems, 2) pH-responsive systems, 3) Specific enzyme-degradable systems, and 4) Systems responsive to specific physiological characteristics.

3.1. Mucous-adhesion systems

Long-term colonization of live bacteria in the host's mucosal layer is crucial for their effects. However, the mucus layer in the host, which contains antimicrobial peptides, acts as a natural barrier against these exogenous microorganisms[124]. Composed of mucins secreted by epithelial cells, glycolipids, immunoglobulins, and electrolytes, the gastrointestinal mucus layer plays a key role in bacterial colonization. Live bacteria have evolved various adhesion strategies, such as fibronectin-binding proteins, exopolysaccharides, and mucus-binding proteins, to adhere to the epithelial cells through receptor−ligand interactions[125,126]. These native adhesion mechanisms are primarily achieved through receptor−ligand interactions. However, these innate adhesion capabilities of these microbes vary among strains, and the composition of the host's microbiota also influences the binding ability. For those live bacteria with weak colonization ability, encapsulation into adhesive polymers is a widely used approach to enhance their effects. These polymers adhere to the mucosal epithelium through mechanisms like mechanical embedding, van der Waals forces, electrostatic attractions, covalent bonds, and hydrogen bonds, allowing bacteria to penetrate the mucosal layer. This approach holds promise for local or systemic therapeutic applications in the gastrointestinal tract[127].

Since the mucus layer of the gastrointestinal tract is negatively charged, cationic encapsulation materials, such as polyethyleneimine[128], poly-lysine, and chitosan[129], can improve the gastrointestinal adhesion properties of bacteria by enhancing electrostatic gravitational attraction, and thus cationization is a commonly used functionalized strategy to improve the retention time of bacteria in the gastrointestinal tract. On the other hand, by chemical modification or covalent conjugation, functional groups with adhesive properties can be added to the live bacteria, which has been widely applied in bacterial surface modification strategies[39,130]. For instance, catechol groups[39], thiol groups, and maleimide groups[131,132] can form stable covalent bonds through Michael addition or Schiff base reactions to enhance the interactions between bacteria and mucins, providing excellent intestinal mucosal adhesion capabilities[53,118,119,131-133]. As depicted in the inset of Fig. 6A, Liu et al.[39] report a double-layer coating strategy employing tannic acid (TA) and enteric L100 for bacterial encapsulation. The strong mucoadhesive capability of the TA layer prolongs the retention time of EcN without compromising the viability and proliferation capabilities of EcN, resulting in superior efficacy of prophylactic and treatment efficacy against colitis. The outer membrane or surface layer proteins (S-layer) of bacteria also contain cysteine residues[134]. Thiolated polymers and maleimides can act as bridges connecting live bacteria and mucus *via* strong covalent bonds, ensuring the positioning of the probiotic delivery system at the target site and enhancing the mucoadhesive ability of live bacteria[118]. Liu et al.[118] designed the

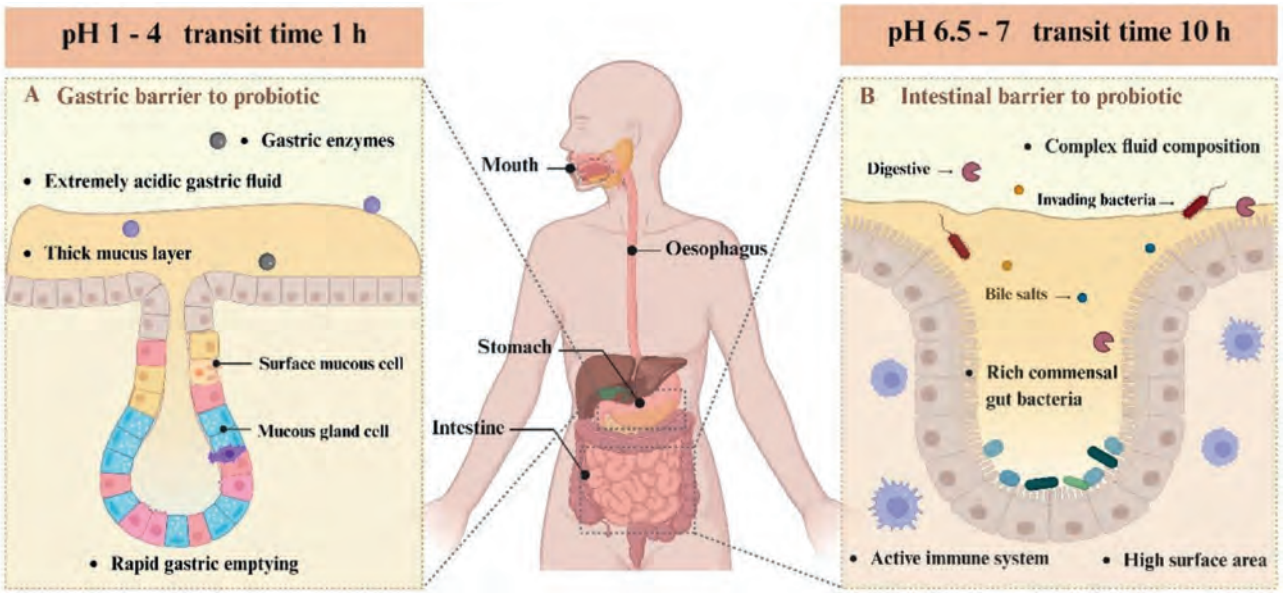

Figure 5 Schematic representation of GIT sections and their pH, transit time and relevant parameters for LTBs delivery.

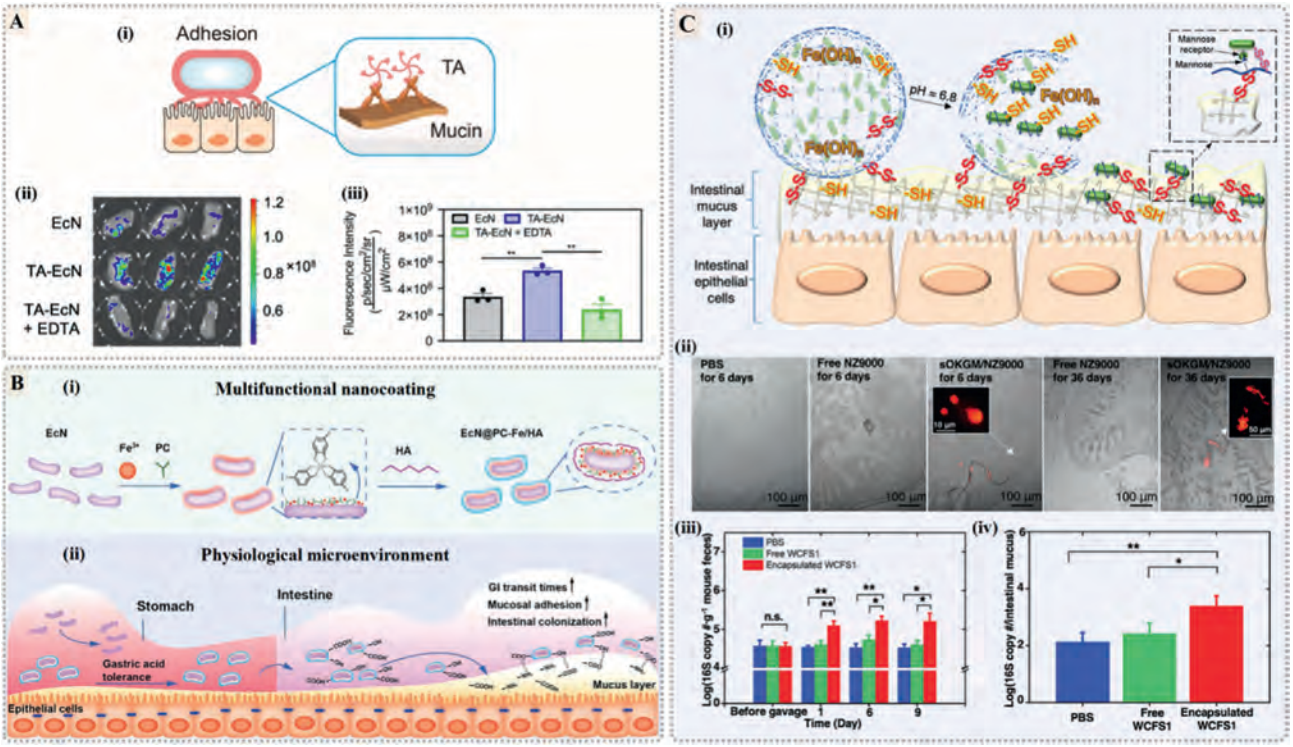

Figure 6 Bacterial delivery mechanism based on gastrointestinal mucosal adhesion. (A) Schematic illustration and characterization of L100-TA-EcN. The exposed TA-EcN adheres on mucosal epithelial layers for prolonging the retention time of bacteria based on the interaction between TA and mucin. Reprinted with the permission from Ref. 39. Copyright © 2021, Elsevier. (B) The process of synthesizing EcN@PC-Fe/HA by layer-by-layer coating strategy using PC, Fe^{III} and HMW-HA. Reprinted with the permission from Ref. 137. Copyright © 2024, John Wiley and Sons. The nanoarmor improved the survival rate of bacteria and enhanced intestinal colonization after oral administration. (C) Thiolated sOKGM polymers mutually interacted with bacteria and the mucus layer *via* disulfide bonds thus enhancing the mucoadhesion of bacteria was enhanced *via* sOKGM bringing linkage. Reprinted with the permission from Ref. 118. Copyright © 2020, John Wiley and Sons.

dual-crosslinked thiolated oxidized konjac glucomannan (sOKGM) microspheres as a potential mucoadhesive oral delivery system for bacteria (Fig. 6C). KGM was selectively oxidized to introduce carboxyl groups through TEMPO oxidation, and cysteines were conjugated to carboxyl groups on OKGM polymers by amide reaction to form sOKGM. Due to large amounts of cysteines on the mucus and surface of bacteria, the sOKGM microspheres mutually interacted with the mucus layer and bacteria, thus improving their mucoadhesion.

Secondly, the binding of lectins to glycans on epithelial cell surfaces through ligand−receptor biorecognition has been widely applied in mucosal drug delivery systems[135]. Lectins specifically bind to glycans, which are highly expressed on mucosal epithelial cells, and this specific binding also enhances adhesion. Petrova et al.[135] designed the expression of two HIV-inhibiting lectins, namely actinohivin (AH) and griffithsin (GRFT), in the probiotic bacterial strains *Lactobacillus rhamnosus* GG and GR-1, for the purpose of delivering them to the gastrointestinal tract and vaginal mucosa, respectively. In addition, utilizing synthetic biology techniques to enhance probiotic adhesion to the mucosa may be a promising approach[93]. Harimoto et al.[93] applied synthetic biology approaches to genetically engineer the biosynthesis of CPS, a naturally occurring extracellular biopolymer, enabling the dynamic regulation of bacterial surface properties through the external inducer IPTG. Probiotics have evolved various adaptive mechanisms to improve their adhesion and colonization abilities in the host[136]. Combination of multiple mucous adhesion strategies can synergistically enhance the mucoadhesive properties, which is significant for the precise targeting and delivery of live bacteria[137] (Fig. 6B).

3.2. *pH-responsive systems*

The live bacteria face harsh challenges during processing, storage, and gastrointestinal conditions. The pH values in the gastrointestinal tract varied from the esophagus to the rectum[138], which can also be altered by pathological conditions such as abnormal metabolic processes and impaired blood perfusion. For example, tumor cells produce lactic acid through glycolysis to obtain energy, but abnormal tumor vasculature hinders the timely removal of lactic acid, resulting in a significantly lower pH of 5.7−7.2 in the tumor tissue microenvironment compared to normal tissues (pH 7.4). Microgels with pH-responsiveness can protect live bacteria against harsh gastric acid and release them responsively in specific intestinal or pathological tissues in a viable form[118,139]. Peng et al.[139] designed a synergetic approach to treat colitis by nanoencapsulation of live bacteria and an anti-inflammatory agent, 5-aminosalicylic acid (5-ASA), within the gastrointestinal microenvironment responsive alginate polysaccharide. Because of acid resistance, the alginate-based coating protected live bacteria from the harsh gastric conditions. The coating could be disintegrated to release bacteria and 5-ASA upon arriving in the intestinal tract, where the pH is normally higher than 5 (Fig. 7A). Luan et al.[140] innovatively prepared a pH-responsive delivery

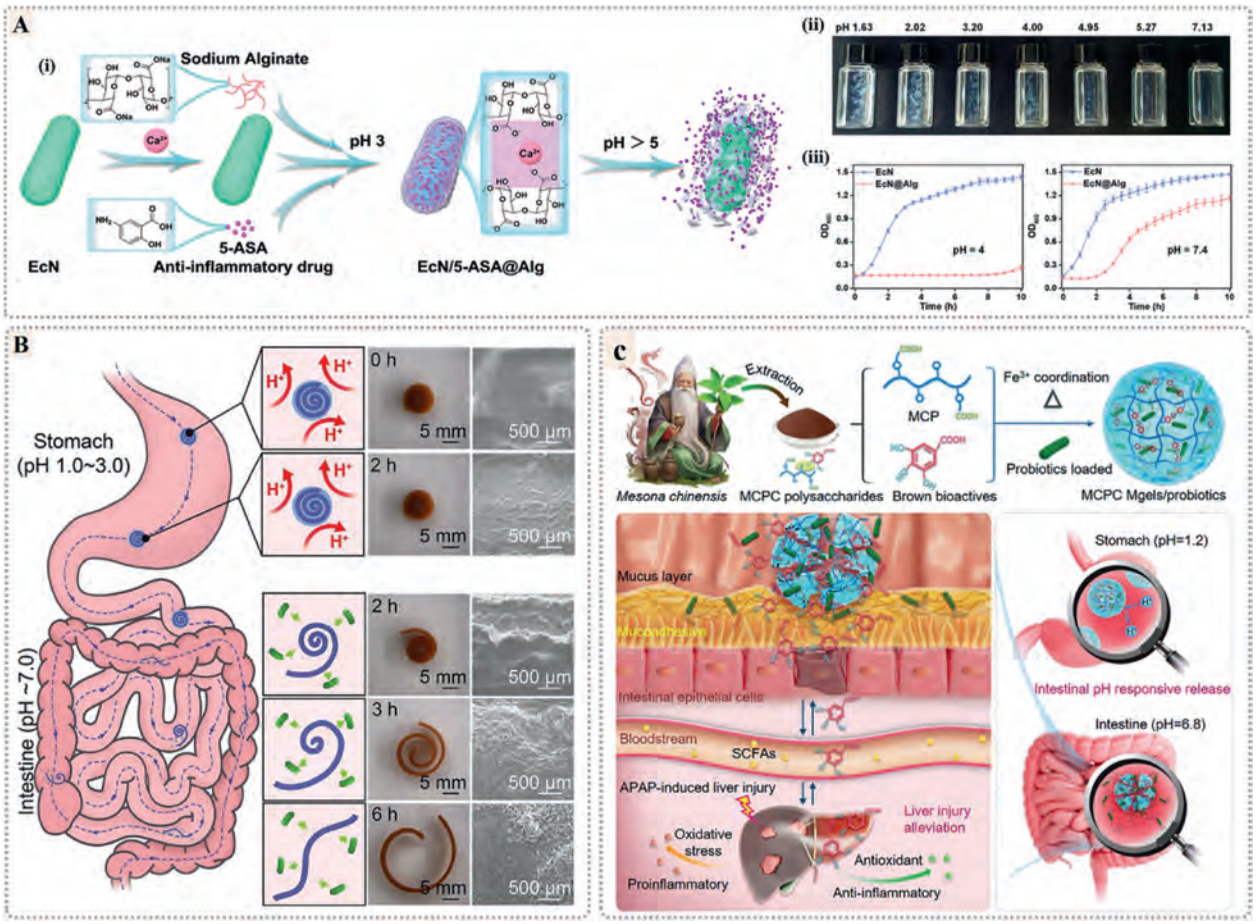

Figure 7 Bacterial delivery mechanism based on gastrointestinal mucosal adhesion. (A) Schematic illustration of the nanoencapsulation of bacteria and an anti-inflammatory agent, 5-aminosalicylic acid (5-ASA), by a gastrointestinal microenvironment responsive alginate coating. Reprinted with the permission from Ref. 139. Copyright © 2023, American Chemical Society. (B) Schematic demonstrating the preparation of the probiotic-loaded wood scroll and the proposed mechanism responsible for the protective and controllable release properties. Reprinted with the permission from Ref. 140. Copyright © 2022, American Chemical Society. (C) Schematic illustration of phenolic-metal coordination framework-stabilized *Mesona chinensis* polysaccharide composite (MCPC) microgels for controlled release the probiotics *Akkermansia muciniphila* (AKK). Reprinted with the permission from Ref. 141. Copyright © 2024, John Wiley and Sons.

scroll using cellulose as the main raw material and sodium alginate as the binder to load *Lactobacillus plantarum* onto flexible wood membranes (Fig. 7B). Similarly, Zhang et al.[141] developed ferric ions cross-linked *Mesona Chinensis* polysaccharide composites (MCPC) microgel with an intestinal responsive and mucoadhesive properties. The abundant carboxyl groups on microgels can absorb and screen the H ions from the stomach acids and protect live bacteria. The iron coordination within the microgels was stable in SGF, because Fe^{3+} induced carboxyl groups (COO^{-}) coordination in microgels are more stable under acidic conditions. When the pH increased, the crosslinker Fe^{3+} formed iron hydroxide, disrupting the crosslinking between Fe^{3+} and carboxyl groups on the microspheres, causing them to rupture and release the bacteria at the targeted sites (Fig. 7C). This scroll maintain stability in the acidic environments (*i.e.*, stomach, pH = 1.0−3.0) and gradually unfolded as the sodium alginate layer dissolved under neutral or weakly alkaline conditions (*i.e.*, intestine, pH ~7.0), enabling continuous and controlled release of the loaded bacteria. However, due to individual physiological differences, dietary conditions, and microbiome variations, the pH values in the body are not stable, leading to limitations in pH-responsive delivery systems, which may fail to trigger the pH-responsive characteristics.

3.3. *Specific enzyme-degradable systems*

The gastrointestinal tract produces a series of specific digestive enzymes, including gastric proteases, pancreatic proteases, amylases, and β-galactosidases, according to the physiological functions required for digestion, etc[138]. By utilizing the specific enzyme-sensitive polymers as delivery carriers, targeted release of live bacteria can be achieved. For example, polysaccharide polymers like linear alginate, chitosan, and guar gum can withstand the harsh environments of the stomach and small intestine, but they can be easily broken down by specific enzymes in the colon. Cheng et al.[142] developed a colon-targeted microcapsule using alginate and protamine, which can protect *Escherichia coli* MG1655 in the stomach and disintegrate layer by layer under the catalysis of trypsin in the intestine (Fig. 8A). The bacterial community in the colon performs various metabolic reactions, including deglucuronidation, decarboxylation, double bond reduction, ester and amide hydrolysis, and dehydroxylation. They

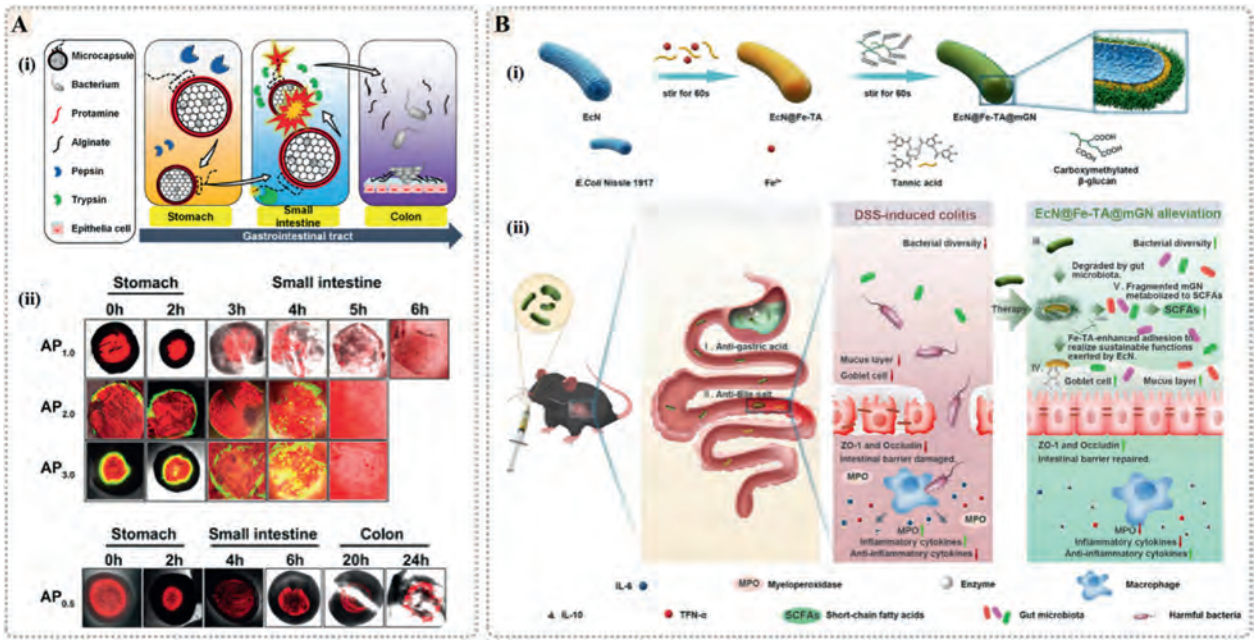

Figure 8 Bacterial delivery mechanism based on specific enzyme-degradable systems. (A) (i) Schematic illustration of the construction of the enzyme-triggered fuse-like microcapsule with multiple alternating layers of alginate and protamine. (ii) The laser confocal microscope images of morphological change of the multilayer microcapsule. Reprinted with the permission from Ref. 142. Copyright © 2021, John Wiley and Sons. (B) (i) Schematic illustration of the fabrication of EcN@Fe-TA@mGN and its alleviation process for DSS-induced colitis in mice. (ii) EcN@Fe-TA@mGN would be degraded by enzymes secreted from the gut microbiota and metabolized to SCFAs to synergize with EcN for the alleviation of colitis. Reprinted with the permission from Ref. 145. Copyright © 2023, American Chemical Society.

meet their energy needs by fermenting undigested dietary fibers and polysaccharides in the stomach and small intestine[143]. For example, polysaccharides, such as chitosan, are mainly digested by glycosidases produced by *Bifidobacteria* and *Bacteroides* residing in the colon. These reactions facilitate the degradation of specific polymers and peptides, which can be used for targeted delivery systems of live bacteria[144,145]. Xie et al.[145] developed a simple yet effective method based on the modified prebiotic to efficiently deliver and colonize probiotics (*i.e.*, EcN). Single EcN was armed with a modified prebiotic-based "shield" composed of an Fe^{3+}−tannic acid (Fe−TA) network layer with another above layer of carboxymethylated β-glucan (EcN@Fe−TA@mGN). Following oral administration of EcN@Fe−TA@mGN, mGN within the "shield" could not only act as a dynamic physical barrier to resist gastric acids and bile salts but also seize the H in the stomach due to the protonation effect for further protection. The underlying Fe−TA layer could be exposed after the degradation of the mGN layer by enzymes secreted from the gut microbiota to aid the colonization of EcN (Fig. 8B). However, these specific enzyme-hydrolyzable materials are generally hydrophilic and tend to absorb water, causing them to swell and release the encapsulated bacteria before reaching the colon. Delivery of bacteria using specific enzyme hydrolysis systems still requires further study of the encapsulation material and thickness in relation to the gastrointestinal environment.

3.4. *Systems responsive to specific physiological characteristics*

Bacteria have shown potential in targeting the tumor microenvironment and treating inflammatory diseases[91,137,146,147]. However, maintaining their stability, site specificity, and therapeutic efficacy is a significant challenge[137]. One possible solution is to design targeted delivery carriers or smart bacteria with different functional modules using bacterial surface coating technology[146]. For instance, Zhu et al.[137] developed an inflammation-targeting armored probiotic called EcN-mCherry@PC-Fe/HA, which base on the overexpressed transferrin in the inflamed colon of inflammatory bowel disease (IBD) patients (Fig. 9A). Firstly, EcN-mCherry@PC-Fe/HA has the ability to bind to the CD44 receptor, allowing it to adhere to inflamed areas. In addition, by incorporating a high-molecular-weight hyaluronan framework into the responsive degradable metal-phenolic networks, researchers could enhance the bacterial resistance to extreme environmental conditions and degrade their nanocoating by responding to transferrin, which ultimately enables targeted delivery and improves the outcome of IBD treatment.

In cancer treatment, the poor accumulation of chemotherapeutic agents within tumors and the complex immunosuppressive microenvironment can reduce the immune response rate and therapeutic efficacy. Over the past decade, it has been widely recognized that bacteria can selectively colonize the immune-privileged tumor core and preferentially grow in the hypoxic and necrotic tumor microenvironment[91]. Researchers have combined the tumor-targeting ability of bacteria with anti-tumor methods such as CAR-T cell therapy and immune checkpoint inhibitors[89,90]. This allows live bacteria to target the release of anti-tumor drugs or immunomodulators within the tumor microenvironment, offering potential for clinical translation[19]. Immunotherapy has proven highly efficacious for certain types of blood cancers, but the lower success rates for solid tumors remain a challenge. As shown in Fig. 9B, Vincent et al.[91] designed bacteria that could home in and colonize solid tumors to improve chimeric antigen receptor (CAR) T cell immunotherapy. The two-step approach involved engineering a nonpathogenic strain of *Escherichia coli*, which delivered synthetic

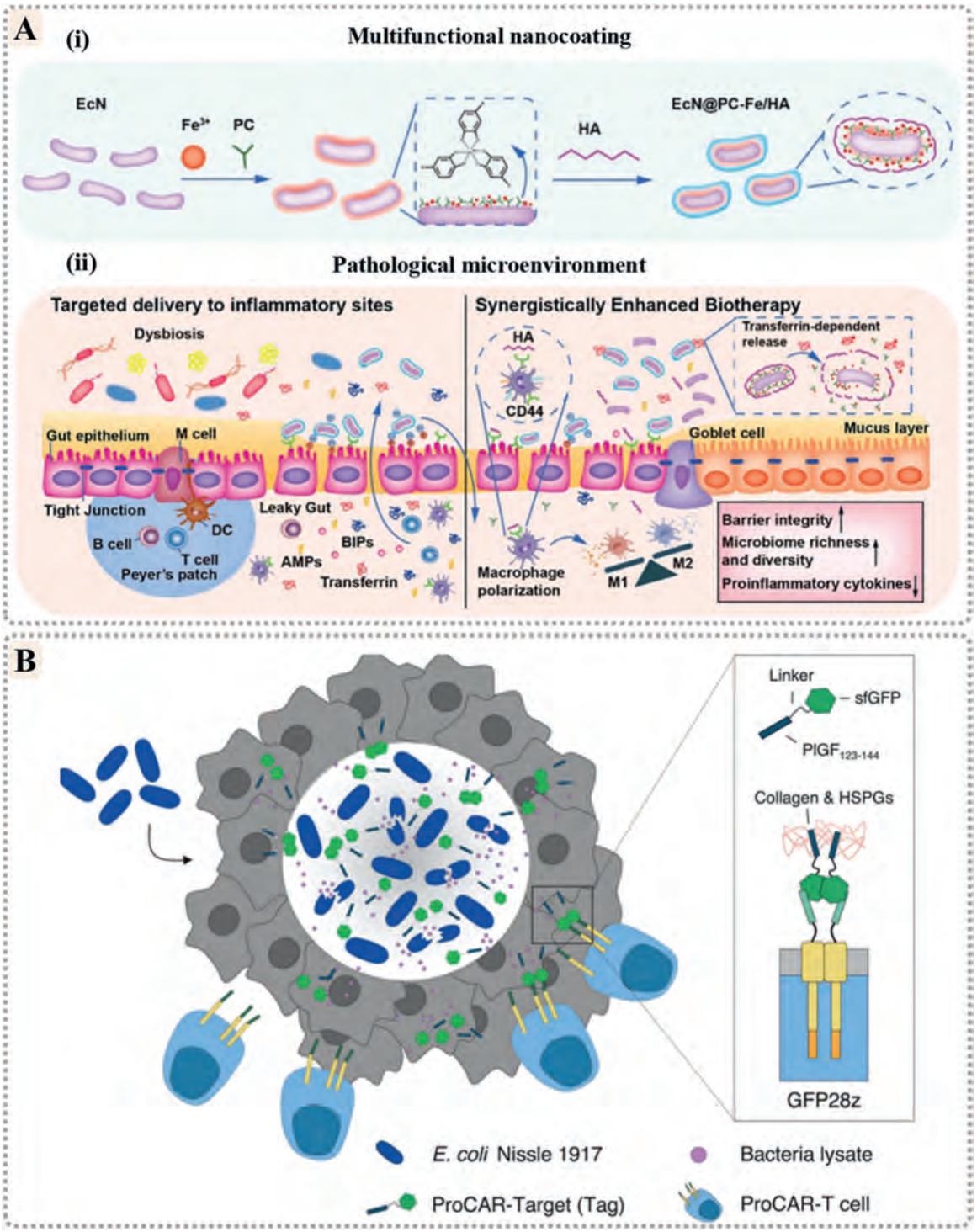

Figure 9 Bacterial delivery mechanism based on specific physiological characteristics of the lesions. (A) A superior armored probiotic (EcN@PC-Fe/HA) with an inflammation-targeting capacity is developed to enhance the efficacy and timely action of bacterial therapy against inflammatory bowel disease. Reprinted with the permission from Ref. 137. Copyright © 2024, John Wiley and Sons. (B) Schematic demonstrating the ProCAR platform, in which synthetic CAR targets (Tags) are produced and released *in situ* by tumor colonizing probiotic bacteria to label ubiquitous components of solid tumors for *de novo* lysis by GFP-CARs (GFP28z). Reprinted with the permission from Ref. 91. Copyright © 2023, The American Association for the Advancement of Science.

antigens to the tumor microenvironment and "tagged" the tumor. They next generated CAR T cells that were programmed to recognize these synthetic antigen tags. When the *E. coli* probiotic was administered, the CAR T cells could be directed to the solid tumors, where they orchestrated tumor cell killing in experimental models of breast and colon cancer.

Using a single environment-responsive probiotic delivery strategy is not a perfect solution. In addition to oral delivery

methods, using "live bacteria" for the treatment of diseases offers unique advantages when delivered through injectable routes. The injected administration of bacteria leads to a relatively high concentration of drugs in the bloodstream, which theoretically makes it easier to determine the therapeutic dosage and potentially improves short-term efficacy and bioavailability[148]. This is particularly beneficial for diseases that require stable therapeutic effects or antibody-based drugs, such as cancer[93,147]. In recent years, there has been a proposal for personalized and precise medication. Combining the aforementioned various precise probiotic delivery strategies and tailoring them to different physiological states within the same individual or different gastrointestinal microenvironments across different individuals would better align with the concept of precision medicine and have more practical significance. Table 2 summarizes the precise release of bacterial surface coatings driven by different strategies.

4. Role of functional bacterial encapsulation strategies in disease treatment

Adding probiotics to reshape the microbial community structure is a crucial strategy for treating dysbiosis-related diseases by regulating the microbial structure and triggering innate immunity[150,151]. As the mechanisms underlying bacterial therapeutic effects have been increasingly elucidated[17,18], LTBs have emerged as advanced biotherapeutics for diverse diseases[11,27,91]. Among the diseases amenable to microbiota-based therapies, gastrointestinal disorders—such as IBD, antibiotic-associated diarrhea, and irritable bowel syndrome—represent primary targets due to the direct involvement of gut dysbiosis in their pathogenesis[39]. In oncology, solid tumors characterized by hypoxic and immunosuppressive microenvironments benefit from bacterial tropism; for instance, the presence of *Akkermansia muciniphila* enhances the efficacy of PD-1 inhibitors in melanoma treatment[152]. In infectious diseases, engineered LTBs can secrete antimicrobial agents to combat pathogens like *Clostridioides difficile*[153]. Additionally, microbiota therapies show promise in autoimmune disorders such as rheumatoid arthritis and multiple sclerosis, potentially mediated through microbiota-driven modulation of T-cell function and immune homeostasis restoration[154].

However, direct oral administration of live bacteria faces challenges from the harsh gastrointestinal environment (strongly acidic pH, bile salts, and digestive enzymes) and specific pathological environments, resulting in the limited bacterial concentrations at disease sites and reduced therapeutic effects[155]. To overcome these challenges, physical encapsulation of LTBs by combining functional materials is an effective approach to compensate for their inherent deficiencies[23,26]. In the field of disease treatment, the effects of adding intelligent coatings to LTBs can be summarized into four categories: 1) harnessing physical barriers; 2) achieving the precise spatiotemporal release targeted at lesions; 3) loading or generating therapeutic agents; 4) triggering the immune microenvironment (Table 3 summarizes the applications of functional encapsulation of bacteria in the treatment of different diseases).

4.1. Harnessing physical barriers

Functional coatings not only help LTBs maintain their viability under the complex gastrointestinal conditions, but also protect them from specific pathological environments caused by diseases[162] and damage from other drugs used during disease

Table 2 Bacterial surface coatings achieve precise release through different mechanisms.

Responsiveness	Delivery location	Probiotic	Advanced functionality	Ref.
Mucoadhesive	Intestine	*E. coli* Nissle 1917	Endows probiotics with superior resistance to the harsh environment of the gastrointestinal tract and enhanced colonization and growth of probiotics in the mucus layer	127
Mucoadhesive	Colon	*B. adolescentis* FS2-3 and *B. subtilis* SN15-2	Improve the survival rate of probiotics and promote proliferation, adhesion, and colonization of probiotics	130
Mucoadhesive/pH responsive	Intestine	Commercial *Bb12*	Both enhanced gastric acid resistance and adhesion colonization at intestine can effectively improve the function of probiotics	118
pH responsive	Intestine	*L. plantarum*	Ensure higher bioactivity as well as prolonged steady release of the nutrient load to the intestine	127
pH responsive	Intestine	*E. coli* Nissle 1917	Upon reaching a location of interest, nanocoatings can be triggered to dissolve *in situ* and subsequently release the bacteria, enabling them to recover their bioactivities as needed	149
pH responsive	Colon	*E. coli* Nissle 1917	Protect and selectively release EcN cells in the GI tract for colonization in IBD treatment	39
Enzyme responsive	Colon	*E. coli* MG1655	Protect the probiotics in the stomach and disintegrate layer by layer under the catalysis of trypsin in the intestine	142
Enzyme responsive	Colon	*L. rhamnosus* GG	Enable an effective local delivery in the cecum and colon without any premature release in the small intestine	144
Pathological environment tendencies	Inflamed-site of inflammatory bowel disease	*E. coli* Nissle 1917	The HA-functionalized nano armor equips the probiotics with inflamed-site targetability through multiple interactions, thus enhancing their efficacy in IBD therapy	137
Pathological environment tendencies	Colon	*L. rhamnosus* GG	Release 5-ASA under azo-reductase to suppress the colonic inflammation and improve the pathological environment	146

Table 3 Application of functionalized bacterial encapsulation in the treatment of different diseases.

Disease	Strain	Materials	Model	Effectiveness	Ref.
Inflammatory bowel diseases	*B. longum*	Artificial-enzyme	Murine models of UC and CD	Reduce ROS levels, inhibit proinflammatory cytokine production, restore the intestinal barrier functions, and increase the richness and diversity of the gut microbiota	19
Inflammatory bowel diseases	*E. coli*	Bionic yeast cell wall	Murine model of DSS-colitis	The high expression of calprotectin at the colitis site triggers the release of tungsten ions through calcium deprivation in CaWO4, thus inhibiting *E. coli* growth by replacing molybdenum in the molybdopterin cofactor	24
Inflammatory bowel diseases	*E. coli* Nissle 1917	PC, Fe^{III}, and HMW-HA	Murine model of DSS-colitis	Efficient inflammatory targeting was achieved through the dual effects of electrostatic interaction and HA-CD44 binding Reshaped the dysbiosis of intestinal bacteria, accompanied by the suppression of acute inflammation and intestinal barrier damage	137
Antibiotic-associated diarrhea	*E. coli* Nissle 1917	Tannic acids and ferric ions	Wistar rats with levofloxacin	Protects both Gram-positive and Gram-negative bacteria against six clinically relevant antibiotics Reduced AAD resulting from the levofloxacin-treatment and improved some of the pre-inflammatory symptoms caused by AAD	147
Tumor	*S. oneidensis* MR-1	Manganese dioxide nanoflowers	Subcutaneous mouse CT26 tumor	Take effect for tumor regression by prominently exhausting extracellular lactate level and inhibiting intracellular lactate production without any molecular drugs participating	156
Tumor	*E. coli* MG1655	Bi_2S_3 nanoparticles	BALB/c mice tumor models	Enhance the radiotherapy sensitivity by triggering the intracellular generation of ROS, coupled with DNA damage Led to significant suppression of breast carcinoma in murine models with reduced side effects	157
Periodontal disease	The strain 25.2.M as *Bacillus* sp.	Chitosan/PEO	*In vitro* agar-well diffusion assay	Against A. actinomycetemcomitans	158
Infected wound	*L. reuteri*	Methacrylate and hyaluronic acid.	A full-thickness cutaneous wound model with *S. aureus* infection	Against harmful bacteria and anti-inflammatory capabilities, promoting infected wound closure and new tissue regeneration	159
Skin injury	*L. reuteri*	Tannic acid, ferric ions, carboxylated chitosan, and oxidized hyaluronan	A full-thickness skin injury model of BALB/c mice	Presented a better performance than the Gel/L in inflammatory regulation, angiogenesis, and tissue regeneration both *in vitro* and *in vivo* in the presence of antibiotics	160
Candida vaginitis	*Lactobacillus*	Peroxidase-like rGO@FeS2 nanozymes	*C. albicans*-infected vaginitis mice	Hardly affected *Lactobacillus*, effectively improving vaginal microbiota and reshaping a healthier vaginal microenvironment	161

treatment[26,163]. Many diseases are characterized by the microenvironments that differ from normal conditions, such as high concentrations of ROS and the hypoxic and immunosuppressive tumor microenvironment (TME)[162], which play crucial roles in disease progression. For instance, IBD often presents a pathological environment with high ROS concentrations, which can oxidize lipids and proteins on the surface of bacteria, causing severe damage to the bacterial cell wall and inactivation[19]. Cao et al.[19] modified the surface of *Lacticaseibacillus rhamnosus* with an artificial enzyme capable of scavenging ROS, creating an anti-inflammatory engineered probiotic (BL@B-SA50). The BL@B-SA50 system provided continuous antioxidant therapy at the

disease site, protecting probiotics from ROS damage while helping to restore intestinal barrier function and increase the richness and diversity of the intestinal microbiota. Furthermore, LTBs are often not administered alone in biomedical applications. In the presence of complex drugs (*e.g.*, antibiotics and adjuvant drugs) and the immune environment in the digestive tract, bacteria may be damaged by other drugs, preventing them from achieving the desired effects[22,164]. Pan et al.[26] utilized the adhesive properties of plant polyphenols on cell interfaces to "dress" bacteria with a "polyphenol nanoarmor" layer. For the cohorts with the administration of levofloxacin, the fecal CFU counts of armored EcC_{tet} flourished rapidly from 2.60×10^6 to 12.56×10^6 CFU/g. This armor absorbed antibiotics surrounding the bacteria through multiple interactions between plant polyphenols and antibiotics, enhancing the antibiotic tolerance of bacteria (Fig. 10A).

4.2. Achieving the precise spatiotemporal release targeted at lesions

Enabling the precise targeting and spatiotemporal release of bacteria or payloads in LTBs has been a continuous focus in the probiotic delivery systems[120,165]. On the one hand, live bacteria have shown potential as intelligent living drugs or drug delivery vectors due to their own genetic manipulation, anaerobic tendency, and immunomodulatory capabilities. On the other hand, we discussed common strategies for using coating materials to facilitate targeted delivery and precise release of bacteria (discussed in Section 3.1). Moreover, various multifunctional nanomaterials have been developed to modify bacteria therapeutics to precisely control the metabolic processes of microorganisms *in vivo*, which ensures that the drugs exert maximum effects upon reaching the target area. Inspired by the non-metabolic life forms like microbial spores, Guo et al.[29] developed a programmable biomimetic dormant system by applying an intelligent nanocoat to therapeutic *Lactobacillus plantarum*. This system is designed for timed activation, with the biomimetic dormant state being "awakened" approximately 2 h after reaching the intestines to synthesize bioactive substances. Compared to unmodified microorganisms, the bioavailability of the biomimetic dormant system in the gut was nearly 3.5 times higher, with enhanced retention maintained for at least 5 days (Fig. 10B). Besides, the ability of certain bacteria to target hypoxic and immunosuppressive environments enables preferential colonization in the core of lesions, achieving site-specific release[29]. In cancer treatment, leveraging the unique physiological metabolism of live bacteria in conjunction with the abnormal metabolism of tumors may provide new

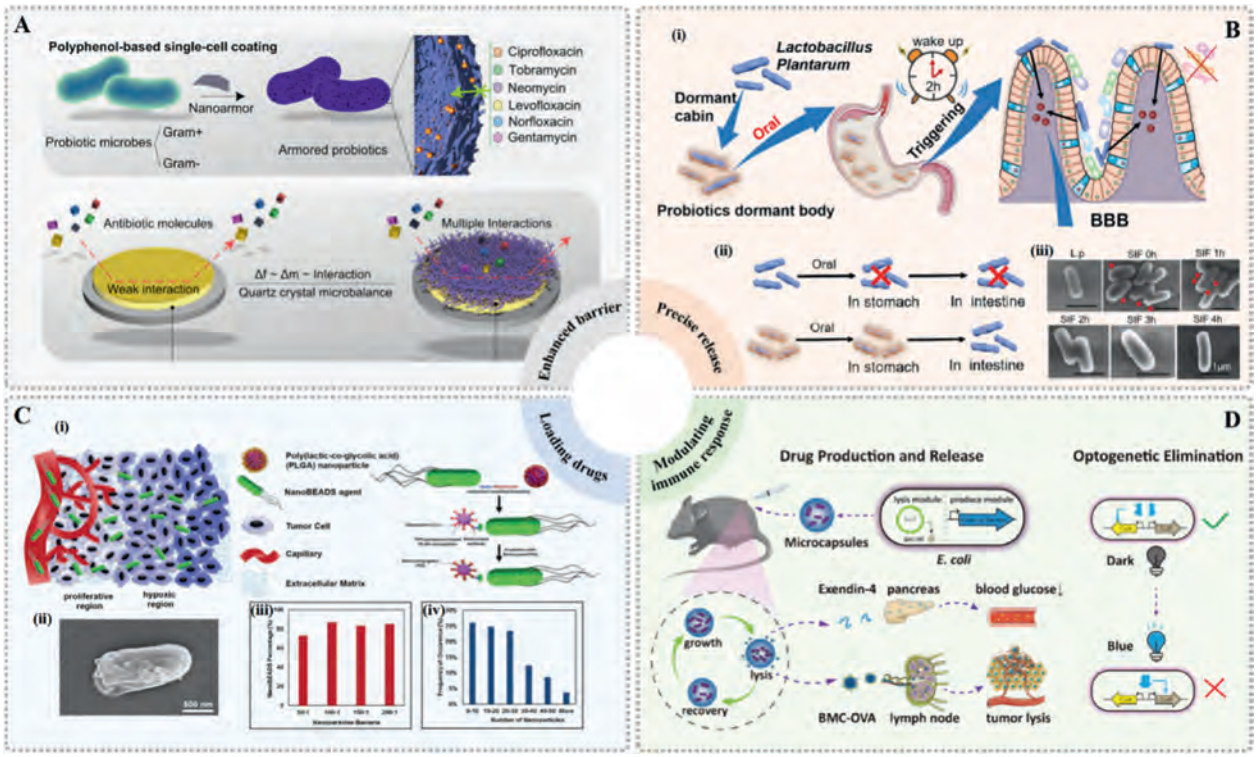

Figure 10 Functional encapsulation strategies of bacteria for disease applications. (A) The nanoarmor enables a rapid and highly biocompatible single-cell encapsulation that protects from a wide range of antibiotics with different molecular structures and properties. Reprinted with the permission from Ref. 26. Copyright © 2022, Springer Nature. (B) Schematic illustration of a timed biomimetic hypnotic body with programmable fate that can predict the metabolic time and location of microorganisms in the body and prevent them from being affected and damaged by the complex biological environment in the body. Reprinted with the permission from Ref. 29. Copyright © 2022, American Chemical Society. (C) Nanoscale bacteria-enabled autonomous drug delivery system (NanoBEADS). Nanoscale drug delivery vectors can be stably bound to the bacteria without toxicity or statistically significant impediment in the bacteria intratumoral transport properties. Reprinted with the permission from Ref. 97. Copyright © 2018, John Wiley and Sons. (D) A self-assembled protein nanovaccine was designed based on engineered bacterial microcomponents (BMC) fused with immunodominant peptide SIINFEKL from model antigen ovalbumin (OVA), hereinafter called BMC-OVA. Reprinted with the permission from Ref. 148. Copyright © 2022, Elsevier.

methods for long-term, painless tumor treatment by using orally administered bacteria as live therapeutics. Over the past decade, researchers have developed various bacteria-based tumor treatment platforms based on specific bacteria's exceptional tumor-targeting ability and natural anti-tumor properties[152,166,167]. This will provide a new perspective on cancer treatment.

4.3. Loading or generating therapeutic agents

Microorganisms are drug production factories that can synthesize a wide range of biologically active substances in a sustained and efficient manner, which makes them very promising tools for the treatment of diseases[152,168]. We have previously discussed that the unique anaerobic tropism of bacteria makes them excellent targeting vectors for anticancer therapies[168]. Drugs can be modified onto the bacterial surface by physical encapsulation, chemical conjugation, or synthetic biology approaches[21], allowing for the autonomous integration of bacteria with therapeutic agents without the need for external driving forces or control inputs. This enables precise drug delivery and assists in bacterial therapy while minimizing systemic side effects[19,20,28,91]. SeungBeum Suh et al.[97] utilized the attenuated *Salmonella Typhimurium* VNP20009 as a "biological postman" to deliver biotin-modified poly (lactic-*co*-glycolic acid) nanoparticles to tumor tissues by conjugating them onto the AV-modified attenuated VNP20009. The results demonstrated that NanoBEADS could enhance the retention and distribution of nanoparticles in solid tumors by up to 100 times without the need for any externally applied driving force or control input (Fig. 10C). Combining synthetic biology and functional material engineering, a new approach enhances the targeted delivery of live bacteria to lesion sites and reduces the complex, overactive inflammatory responses nearby[19]. Cao et al.[19] employed synthetic biology to genetically engineer probiotics to express antioxidant enzymes and used material design to distribute the antioxidant mimic enzyme (B-SA) on the probiotic surface. This approach avoids bacterial lysis and provides stable scavenging capabilities against multiple ROS, effectively addressing the complex IBD environment.

4.4. Triggering the immune microenvironment

In recent years, functional materials, particularly those leveraging nanobiotechnologies, have emerged as a promising strategy to enhance immune activation and tumor targeting[51,169,170]. These materials improve the delivery and efficacy of immunotherapeutic agents by utilizing the unique properties of nanomaterials. The integration of nanobiotechnologies with live bacterial therapies has shown significant potential in enhancing therapeutic outcomes. Properly coordinating the relationship with the host's immune system is a fundamental responsibility of LTBs. On the one hand, the host's immune system acts as an essential barrier against exogenous microorganisms, with macrophages in the intestinal mucosa maintaining their bacterial phagocytic and sterilization abilities[171]. Surface coating techniques can provide bacteria with a "cloak of invisibility"[42], where the cell membranes of human blood cells are extracted and used to envelop the bacteria, presenting a simple strategy for bacterial camouflage. This method achieves two goals simultaneously: enabling bacteria to effectively escape immune surveillance, thereby significantly increasing their accumulation at tumor sites. On the other hand, bacterial infections can promote tumor immunogenicity, further inducing effective anti-tumor immune responses. Oral tumor vaccines based on engineered probiotic vectors are non-invasive alternatives for eliciting potent anti-tumor immune responses[42]. Therefore, in addition to evading clearance by the host's immune system, live microbial therapeutics need to induce immune responses when acting as vaccines. Surface coating strategies can enable live microbial therapeutics to accumulate in the intestinal lymphatic system or cross the intestinal barrier, activating the host's immune system and facilitating effective uptake and presentation by immune cells[117]. Han et al.[148] successfully activated the immune system and exhibited preventive effects against B16-OVA tumors in mice using an OVA subunit vaccine based on the bacterial minicell carrier (BMC) (Fig. 10D). Besides, Lin et al.[117] successfully encapsulated *Escherichia coli* Nissle 1917 with yeast membrane vesicles (YMs) to create a novel encapsulated bacterium, EcN@YM. The experiments showed that after oral administration for 4 h, the accumulation of EcN@YM in the Peyer's patches was nearly eight times that of the unencapsulated EcN. Moreover, the β-glucan present on the yeast membrane of EcN@YM can be recognized by the Dectin-1 receptor on M cells, thereby promoting the uptake by M cells. This characteristic further triggers a strong mucosal immune response, which helps maintain the healthy homeostasis of the gut microbiota under perturbation factors such as pathogenic bacterial infection and intestinal damage, and protects the intestinal barrier function.

5. Conclusions and outlooks

Currently, the advantage of bacterial therapy lies in its inherent motility and growth potential, allowing targeted bacteria to specifically enter lesion sites and stably proliferate, effectively overcoming the inability of existing clinical drugs to localize to potential metastatic lesions (Fig. 11). The surface decoration of bacteria combines chemical, physical and bioengineering and other multi-methods to integrate the advantages of materials and bacteria, which not only endows the bacteria with new functions of responding to the pathological environment intelligently and releasing drugs accurately, but also coordinates the interrelationships with the immune system, which greatly broadens the means and modes of bacterial therapeutics. Functionalized encapsulation strategies of bacteria achieve more advanced synergistic effects and application scope in bio-imaging, diagnosis to the treatment of various diseases[46]. It lays the theoretical foundation and new development direction to promote the development of intelligent live bacterial therapeutics. The market for live bacterial supplements or therapeutics is showing significant growth, yet there are still key scientific questions to be addressed to achieve translation from laboratory to clinical applications.

(1) Live bacterial therapeutics face three major bottlenecks in clinical translation: unstable large-scale production, insufficient stability during long-term storage, and low efficiency of biological agent application[154,170,172]. To address these challenges, multidimensional synergistic optimization strategies can be implemented. 1) Innovative development of multimodal delivery systems. First, physical techniques such as layer-by-layer encapsulation can shield live bacteria with polymers, enabling them to withstand the harsh gastrointestinal environment and promoting intestinal colonization. However, the mechanical barriers of traditional encapsulation materials may impede nutrient absorption and bacterial signaling. In this regard,

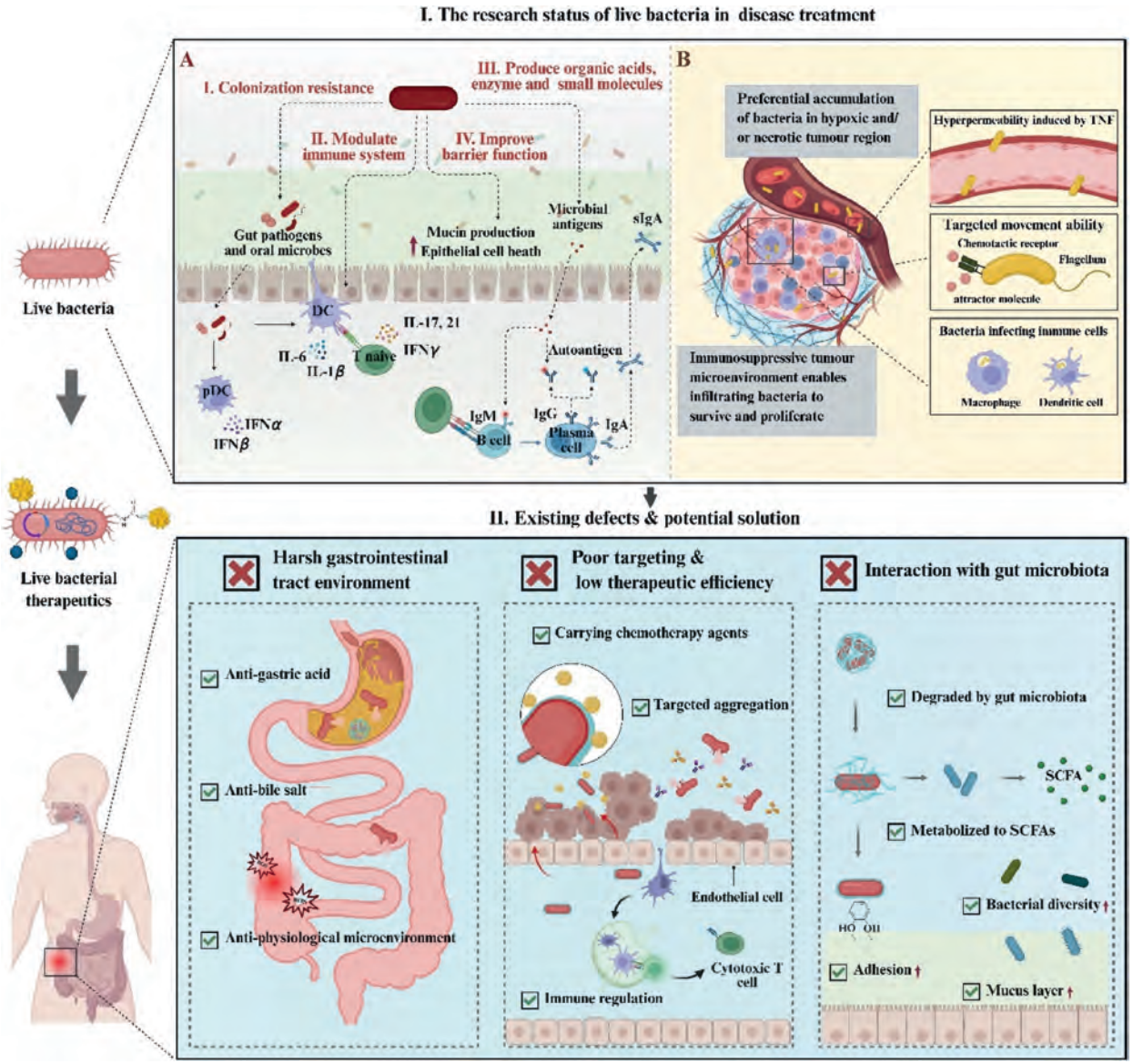

Figure 11 Illustration depicting the summary and future development directions of live bacterial therapeutics. I. The research status of live bacteria in disease treatment. (A) The mechanisms of live bacterial therapeutics in disease treatment. (B) Mechanisms of live bacteria as carriers of therapeutic agents. Tumors have a characteristic microenvironment that markedly diverges from the physiological state of most non-malignant tissues and renders them a preferential target for certain bacteria, which can be exploited for bacteria-mediated cancer immunotherapy. II. Existing defects and potential solution for bacteria as therapeutic agents. Key areas of focus include enhancing resilience against the harsh gastrointestinal tract environment, improving targeting and therapeutic efficiency of live bacterial therapeutics, and understanding the interactions between gut microbiota. These advancements aim to optimize the efficacy and applicability of live bacterial therapies in clinical settings.

programmable encapsulation systems based on synthetic biology can dynamically regulate the synthesis of bacterial surface polysaccharides, not only enhancing immune evasion capabilities but also achieving controllable clearance after treatment, significantly increasing the tolerated dose and therapeutic effect[173]. The development of efficient delivery systems needs to balance immune evasion and the maintenance of metabolic activity[137]. 2) Dynamic regulation of *in vivo* functional activity. LTBs need to be precisely activated in the microenvironment of the lesion. By integrating hypoxia or reactive oxygen species (ROS)-responsive promoters, site-specific gene activation systems can be designed to release anti-inflammatory factors or therapeutic proteins only under specific conditions, avoiding off-target effects[93]. 3) Synergistic enhancement of functional materials and live bacteria. New functional materials, such as photoresponsive, thermoresponsive, and magnetic materials[174,175], can achieve dynamic regulation, precise targeting, and synergistic enhancement of probiotic activity, providing new technological avenues for individualization and precision medicine. For example, magnetically guided targeting technology can increase the delivery efficiency of live bacteria several-fold, while temperature-controlled hydrogels can achieve programmed sustained

release of drugs. 4) Process innovation for large-scale production. As dynamic biological entities, bacteria need to have stable, standardized, and controllable large-scale production methods established for clinical translation. The integration of novel technologies such as 3D printing[102] and microfluidics[103] into probiotic encapsulation has led to a more miniaturized, high-throughput bacterial encapsulation from design to production, intelligence[106,107] and biomimetic chemistry[102] among other features[176,177]. In addition, some new technologies encapsulate and deliver multiple interactive ingredients in such a way that they do not interfere with each other, offering the possibility of incorporating prebiotics and other functional materials[103,104]. However, functional materials cannot co-proliferate with bacteria[97], and more critical parameters are still needed for the study of whether the overloading of materials affects the metabolism and function of engineered bacteria as well as the targeting and therapeutic effects of diseases[70].

(2) In biomedical applications, live bacterial therapeutics require pharmacokinetic and drug performance evaluation based on specific target disease models. However, the modeling of different target diseases and the human intestinal environment is not yet fully developed, and there is a lack of uniform and authoritative specifications for the drug performance evaluation of live bacterial therapeutics. In addition, live bacterial therapeutics are often used in combination with other drugs in disease treatment applications, and their interactions deserve further study and evaluation.

(3) The biosafety issues of live bacterial therapeutics in clinical applications warrant significant attention. Firstly, at the production process level, live bacterial preparations cannot utilize traditional sterilization methods such as high-temperature sterilization or membrane filtration, resulting in a significantly higher risk of pathogenic contamination compared to conventional biological products. During clinical application, uncontrolled proliferation of live bacteria in the host may not only trigger excessive immune activation (*e.g.*, cytokine storms) but also pose systemic infection risks in immunocompromised populations[178]. Studies indicate that functional encapsulation or genetic engineering of live bacteria can retain immunostimulatory efficacy while substantially reducing infection risks[179,180]. Notably, the gut microbiota serves as a potential reservoir for antibiotic resistance gene transmission, and live bacterial therapies may exacerbate the spread of antibiotic-resistant bacteria[110,111]. Therefore, strict screening for resistance gene carriage during strain selection and CRISPR-based gene editing to knockout mobile genetic elements are imperative. A preclinical evaluation system integrating metabolomics and immunomics should be established to monitor colonization dynamics, immunogenicity profiles, and horizontal gene transfer risks. Although genetic editing technologies have significantly enhanced the controllability of live bacterial therapies, standardized clinical safety evaluation criteria remain lacking. It is recommended to establish a three-tiered assessment system encompassing genetic stability, ecological safety, and dose-effect relationships[181]. Thus, in addition to continuous innovation to overcome technical bottlenecks, a comprehensive regulatory system must be established to support and advance clinical translation and industrialization in this field.

(4) Improving the personalization and clinical applicability of live bacterial therapies is also a key concern. Personalized engineered bacteria can be tailored to the genetic and immune profiles of both patients and bacterial strains, representing a pivotal direction in live bacterial therapeutics. While personalized microbiome profiling is emerging as a cornerstone of precision medicine with demonstrated efficacy across conditions from cancer to infectious diseases, overcoming technical and clinical translation challenges demands interdisciplinary collaboration. Below, we propose three strategic recommendations for advancing precision live biotherapeutics: 1) Multi-omics integration and AI-driven predictive models. By integrating multi-dimensional data from a patient's microbiome, metabolome, and host genome, a machine learning-driven therapeutic response prediction system can be constructed. Analyzing the composition and functional characteristics of a patient's microbiota enables intelligent strain selection, dose optimization, and dynamic therapeutic effect monitoring. Multi-omics technologies (such as metagenomics and metabolomics) can be used to analyze the composition and functional characteristics of a patient's microbiota, enabling the identification of key microbial imbalances or metabolic pathway abnormalities[182]. In melanoma, elevated abundances of *Ruminococcaceae* and *Akkermansia muciniphila* correlate with enhanced PD-1 inhibitor efficacy. Interventions like fecal microbiota transplantation (FMT) or oral probiotic supplementation (*e.g.*, *A. muciniphila*) can remodel gut microbiota in non-responders, significantly improving immunotherapy response rates[183,184]. 2) The combination of molecular biology techniques and bacterial surface engineering enables precise regulation. Microbial metabolites, such as short-chain fatty acids, can influence disease progression by modulating host immunity or causing genotoxicity[185]. By integrating metabolomics analysis with synthetic biology modifications, it is possible to identify subgroups with metabolic deficiencies and design precise supplementation plans. Moreover, by employing functional encapsulation strategies for bacteria, we can integrate multiple probiotics, enzymes, and nutritional factors, enabling "multi-target" and "personalized" precision nutrition or therapy. 3) Host−Microbiome Interaction Optimization Host genetics, dietary patterns, and medication history (*e.g.*, antibiotics) critically shape microbiome intervention outcomes[186]. Antibiotics deplete *A. muciniphila*, leading to PD-1 therapy failure, while its supplementation reverses immunosuppression[184]. Personalized microbiome-guided approaches thus represent a paradigm shift, moving beyond one-size-fits-all therapies to fully harness live biotherapeutics' potential. The integration of molecular biology, bacterial engineering, and AI-driven analytics with personalized microbiome profiling heralds a transformative era in precision medicine. Sustained innovation in synthetic biology, regulatory frameworks, and cross-disciplinary collaboration will be essential to realize fully personalized bacterial therapies.

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Author contributions

Ying Zhang: Writing-original draft. Yuwei Wu: Writing-review & editing. Xinyu Zhao: Writing-review & editing. Qinghua Ye: Validation, conceptualization. Lulu Cao: Validation, conceptualization and visualization. Ming Liu: Conceptualization, visualization. Bao Gao: Conceptualization. Qinya Niu: Validation, conceptualization. Nuo Chen: Conceptualization. Zixuan Duan: Conceptualization. Yu Ding: Validation. Juan Wang: Validation. Moutong Chen: Validation, conceptualization. Ying Li: Writing-review & editing, Supervision. Qingping Wu: Writing-review & editing, supervision.

Conflicts of interest

The authors declare no conflicts of interest.

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