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REVIEW

Modified probiotics and the related combinatorial therapeutics

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Abstract Probiotics have shown excellent application prospects in preventing and treating many diseases. However, their sensitivity to the harsh environment *in vivo* always leads to a massive loss of viability and insufficient therapeutic effect. Fortunately, modified probiotics have emerged and provide multiple possibilities for their use in various diseases. Modification not only endows probiotics with extra capacity to resist severe environments but also gives them exogenous characteristics, such as prolonged retention time and improved therapeutic effects. Modified probiotics could combine with other therapies, which has opened up new avenues to enhance the efficacy of probiotic-based therapy. In this review, we have summarized the current physicochemical and biological modification strategies of probiotics. In addition, the progress of research on probiotic-based combination therapy has also been extensively reviewed, which contributes to the enhanced delivery of probiotics or other active constituents and provides new ideas for disease treatment, bioimaging, and diagnosis.

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1. Introduction

Probiotics are defined as live microorganisms that, when given in adequate amounts, confer a health benefit on the host[1]. The probiotics market is projected to reach USD 105.7 billion by 2029, growing atan 8.2% CAGR from USD 71.2 billion in 2024[2]. Many clinical trials have shown that probiotics are pivotal in human health, disease prevention, and treatment. Traditional probiotics, such as strains of *Lactobacillus* and *Bifidobacterium*, have shown efficacy in treating diseases like irritable bowel syndrome (IBS)[3], inflammatory bowel disease (IBD)[4], and certain infections[5,6]. Furthermore, probiotics also contribute to tumor prevention and treatment by enhancing immunity, inhibiting carcinogens, promoting beneficial metabolites, suppressing harmful gut bacteria, and altering the tumor microbiome[7,8].

Even though probiotics have shown many exciting achievements, the clinical translation of probiotic-based therapy remains challenging[9]. This is primarily due to their high sensitivity to environmental changes. For instance, probiotics taken orally may face low colonization and survival rates, whereas probiotics injected intravenously are subject to clearance by the circulatory system[10]. As a result, using probiotics alone may not always yield the desired therapeutic outcomes.

Recent advancements have focused on developing modified probiotics to overcome these limitations. Modifications are currently divided into physicochemical and biological categories[11]. After modification, probiotics can be equipped with molecules like medicine and antibodies or protective coatings, such as hydrogel networks and nanoparticles[12,13]. The methods above could protect probiotics and endow them with new functions simultaneously.

With the help of various material modifications, probiotics may acquire increased resistance to the harsh gastrointestinal environment, greater oral absorption efficiency, improved ability to adhere to mucosal surfaces, and enhanced colonization of the intestines[14,15]. Those new functions enable the integration of probiotics with other therapeutic approaches for treatment. Combined therapy leverages the advantages of probiotics to deliver therapeutic agents directly to specific disease sites while also optimizing the therapeutic efficiency of probiotics themselves. In addition, integrating probiotics with other therapeutic approaches has opened up new avenues for disease imaging[16], diagnosis[17], and treatment[18]. Combining probiotics with photosensitizers and immunoactivators forms an immunoreactive nanosurface on the probiotic. This hybrid system provoked robust anti-tumor immunity, and the combination therapy significantly inhibited tumor growth and extended the survival of animals. Moreover, genetic engineering has been widely used to create therapeutic agents in various probiotics[19,20]. Gene editing probiotics like *in vivo* "therapeutic factories" could produce edited biotherapeutics precisely at disease lesions for improved therapeutic efficacy[21-23]. These synergistic approaches can potentiate the therapeutic effects of probiotics, offering a multifaceted strategy to tackle complex diseases.

This review article discusses physicochemical and biological modification methods based on various surface structures on probiotic cells. After introducing new functions, we then outline the use of the modified probiotics in combination therapy and other applications in detail (as shown in Fig. 1). We anticipate that this review article will foster the development of innovative strategies for creating advanced probiotic-based therapies through modification.

2. Probiotics modification

Probiotic modification involves altering or coating the surface of probiotics using physical, chemical, or biological techniques to introduce new functionalities distinct from their original properties. In probiotics, surface structures and characteristics are critical for cell communication and interactions with surrounding environments[24]. Elements such as surface charge, chemical groups, proteins, and antigens significantly influence key physiological functions, including adhesion, proliferation, and differentiation. Modifying the surface of probiotics can also trigger specific physiological signals and responses.

The primary aim of probiotic modification is to reduce potential side effects while enhancing their functionality and broadening their applications by refining surface characteristics and facilitating beneficial interface interactions. As our understanding of probiotic surface properties deepens and interdisciplinary research advances, various physicochemical and biological methods have emerged as effective approaches for modifying probiotic surfaces.

2.1. *Physicochemical modification*

The surface of probiotics mainly comprises cell walls and exterior structures, such as flagella and pili. Probiotics' multilayer cell wall structure comprises polysaccharides, proteins, and lipids. Those components are rich in diverse functional groups (*e.g.*, hydroxyl, carboxyl, amine, and free thiol groups), offering modifiable reaction sites for chemical modification on the surface of probiotics[25,26]. Multiple chemical modification methods have been developed based on these structural characteristics. Many materials that contain amino or carboxyl groups can be modified into probiotics with the aid of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC)/NHydroxysuccinimide (NHS), those can bond with probiotics together. Additionally, functional polymers can be treated using bioorthogonal chemistry to enable the spatiotemporal delivery of bacteria by a click reaction with the target location[27]. There are different modification methods for individual probiotics and multiple probiotics. For individual probiotics, materials form as coating or conjugators on the surface of probiotics. As for multiple probiotics, encapsulating is the primary method of modification. The physicochemical modification examples are listed in Table 1[29-63].

2.1.1. *Single-cell modification*

The individual probiotic coating is a single-cell nano-encapsulation technology in which a coating method imparts non-inborn or natural external features to live cells. Because of the complete coverage of a coating on the surface of probiotics, individually coated probiotics typically exhibit enhanced bioavailability, a controlled release profile, and improved pharmaceutical effects in comparison with uncoated probiotics[28].

2.1.1.1. Surface coating on single cell. Metal-polyphenol network coating: Polyphenols are natural plant chemicals abundant in fruits, vegetables, and cereals. Polyphenolic biomolecules share the same structural characteristics: the phenolic hydroxyl groups. The abundance of phenolic hydroxyl groups provides polyphenols with firm adherence to various surfaces.

Interestingly, metal ions are usually introduced to the surface of biomaterials *via* phenolic hydroxyl groups, forming metal-phenolic networks (MPNs). Based on their above features,

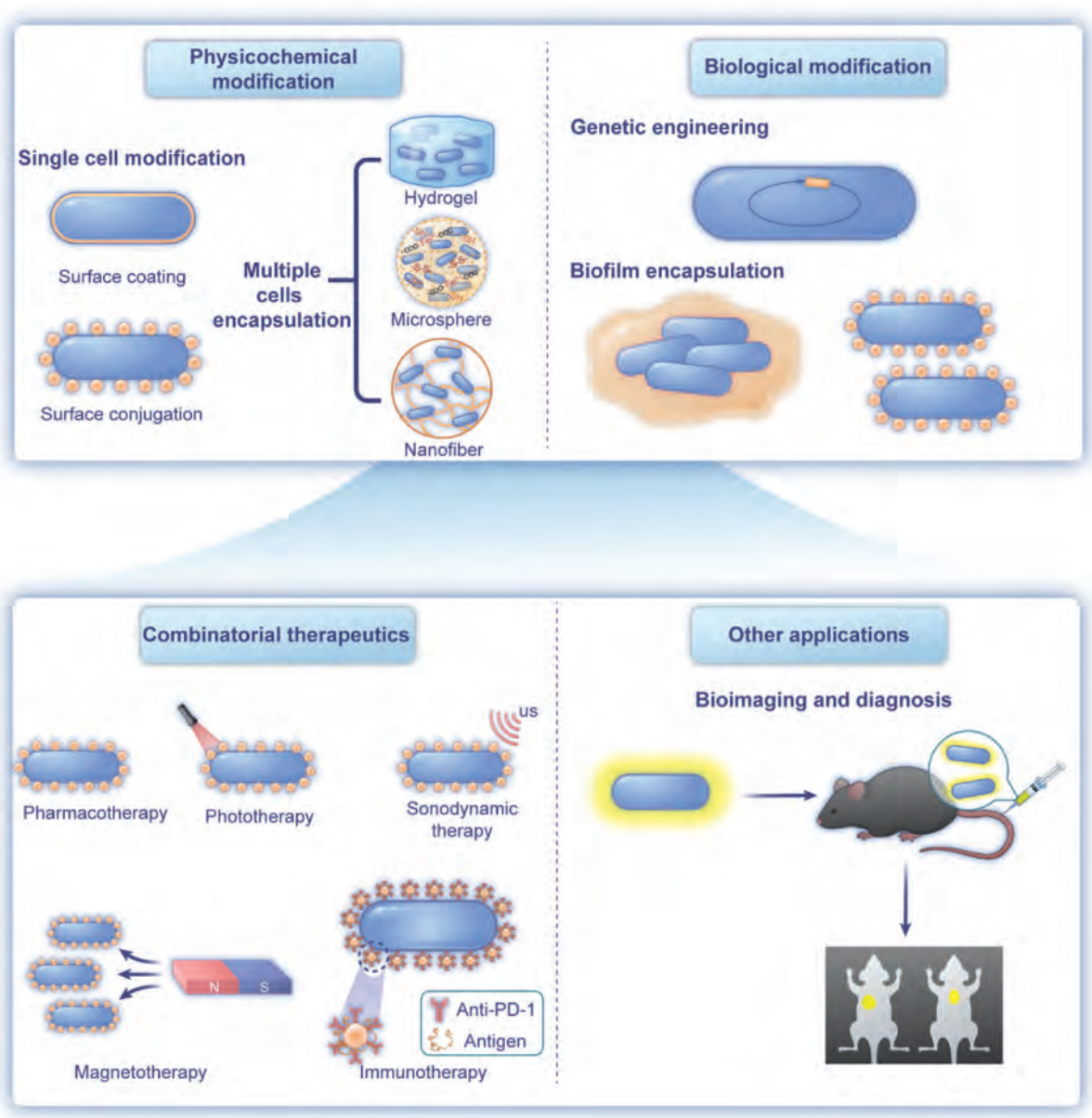

Figure 1 Schematic illustration of the modification of probiotics and the related combinatorial therapeutics.

polyphenols can be used to coat probiotics. Through the self-assembly of metal and polyphenol, Fe-TA coated probiotics (*Lactobacillus reuteri*, *L. reuteri*@FeTA) were then placed into an injectable hydrogel made of carboxylated CS and oxidized hyaluronic acid to create Gel/L@FeTA. To expedite wound healing, the hydrogel efficiently enhanced collagen deposition surrounding the wound-regulated inflammatory markers and increased the expression of angiogenesis-related proteins[64]. Based on the Fe-TA prebiotic "armor," Xie et al.[30] constructed the EcN@Fe-TA@mGN to form a dynamic barrier, which enhanced the EcN colonization in the intestine. Furthermore, the combination of carboxymethylated β-glucan with EcN was found to have a synergistic effect in regulating the gut microbiota and stimulating the production of short-chain fatty acids (SCFAs). In a related study, Pan et al.[29] employed a single-cell coating strategy using TA and trivalent ferric ions, referred to as "nano-armor", to shield bacteria from the effects of six clinically significant antibiotics.

Apart from the typically utilized Fe^{3+}, the metal calcium ion Ca^{2+} can also chelate with TA. As shown in Fig. 2A, Yang et al.[31] created a super probiotic (EcN@TA-Ca^{2+}@Mucin) layer-by-layer coat of EcN with tannins and mucins. It was additionally demonstrated that mucin increased probiotic colonization and development of probiotics in the mucus layer through interaction with mucus, providing better resilience to the challenging gastrointestinal environment and robust adherence to the intestine. Furthermore, through the scavenging of reactive oxygen species (ROS), the EcN@TA-Ca^{2+}@Mucin complex had the potential to significantly reduce inflammation, alleviate the adverse consequences of bacterial translocation in inflammatory bowel disease, and enhance the diversity and abundance of intestinal microbiota.

Polymer coating: There are two main polymer categories: natural and synthetic. Due to their biocompatibility and simple formulation parameters, polymers are widely used as drug-delivery vehicles[65]. In the case of probiotics, polymers can be

Table 1 Modification of probiotics.

Modification type			Probiotic	Modified material	Bond/modification method	Ref.
Physicochemical modification	Surface coating on single cell	Metal-phenolic	EcN	Fe^{3+}, TA	Hydrophobic interaction, electrostatic interaction, Hydrogen bonding	29
			EcN	Fe^{3+}, TA, mGN	Hydrogen bonding	30
			EcN	Ca^{2+}, TA, mucin	Hydrogen bonding, Disulfide bonding, Hydrophobic interaction	31
		Polymer coating	EcN	PDA, CS	Covalent or hydrogen bonds	32
			EcN	pNE, HPN	Oxidative self-polymerization	33
			EcN	CS, ALG	Electrostatic self-assemble	34
		Lipid coating	EcN	DOPA, cholesterol	Biointerfacial supramolecular self-assembly	35
			LCB	Liposomes,	Self-assembly	36
		Mineral coating	BF839	$CaCO_3$, PVP	Electrostatic interaction −mediated biointerface mineralization	37
		Other coating materials	EcN	SiH@TPGS-PEI	Electrostatic interaction	38
			Lac	DOPA, PA, HA	Amide bonding	39
			EcN	TA, F68	Self-assemble	40
			EcN	5-ASA, Ca^{2+}, ALG	Electrostatic interactions, Ionic bonds	41
			Li01	CS, ALG	Electrostatic attraction	42
	Surface conjugation on single cell	Non-covalent	EcN	PTX, HPB	Hydrophobic interaction, electrostatic interaction	43
			E. hallii	Fe-DOX	Supramolecular self-assemble	44
		Covalent	*C. butyricum*	MGEM	Amide bond	45
			BL	Fe SA	Click reaction	46
	Multiple cells encapsulation	Hydrogel	LGG	Bentonite/ALG nanocomposite hydrogels	Electrostatic interactions, van der waals interactions	47
			L. Lactis	CS, PEGDA	Amide bond	48
			LP	BSP, CS	Schiff-base reaction	49
			L. rhamnosus	Thiolated HA	Disulfide bonds	50
		Microspheres and microcapsules	NZ9000	sOKGM	$COO^{-}-Fe^{3+}$ coordination, disulfide bonds	51
			LDB, LGG	Calcium ALG microsphere, CS, Eudragit L100-55	Electrostatic interactions	52
			E. coli MG1655	ALG, protamine	Electrostatic interactions	53
			L. plantarum	Casein-CS	Ionic bonds, Schiff-base reaction	54

Table 1 (*continued*)

Modification type			Probiotic	Modified material	Bond/modification method	Ref.
			L. plantarum, *L. paracasei*	Ca^{2+}, ALG, Sucrose	Ionic bonds	55
			FS2-3, SN15-2	CMC−SH	Disulfide bonds	56
		Nanofiber	LGG	Pullulan nanofibers, electrospun PLGA	Electrostatic interactions	57
			Bacillus	PEO, PEO/ALG	Electrostatic interactions	58
Biological modification	Genetic engineering		*Lactobacillus*	−	Gene editing	59
			L. lactis	−	Gene editing	60
			Lactobacillus casei	−	Gene editing	61
	Biofilm encapsulation		EcN	YM	Physical extrusion	62
			Bi	Biofilm produced by *Lr*	−	63

ALG, alginate; *Bacillus*, *Bacillus* strains; BF839, *Bacteroides fragilis*; Bi, *Bifidobacterium bifidum*; BL, *Bifidobacterium longum*; BSP, *Bletilla striata* polysaccharide; Ca^{2+}, calcium ions; $CaCO_3$, calcium carbonate; *C. butyricum*, *Clostridium butyricum*; CMC-SH, thiolated carboxymethyl cellulose; CS, chitosan; DOPA, dopamine hydrochloride; DOX, doxorubicin; EcN, *Escherichia coli Nissle*; *E.coli* MG1655, *Escherichia coli* MG1655; *E. hallii*, *Eubacterium hallii*; Fe^{3+}, ferric ions; Fe-DOX, DOX-loaded iron-polyphenol nanoparticles; Fe SA, iron single-atom catalyst; FS2-3, *Bifidobacterium adolescentis* FS2-3; F68, poloxamer 188; HA, hyaluronic acid; HPB, BAY-876 bound human serum albumin nanodrugs; IBD, inflammatory bowel disease; Lac, *Lactobacillus acidophilus*; LDB, *Lactobacillus delbrueckii* subsp. *Bulgaricus*; LGG, *Lactobacillus rhamnosus GG*; Li01, *Ligilactobacillus salivarius Li01*; *L. lactis*, *Lactococcus lactis*; *L. paracasei*, *Lactobacillus paracasei*; LP, *Lactobacillus plantarum*; *L. plantarum*, *Lactobacillus plantarum*; *Lr*, Lactobacillus royale; *L. rhamnosus*, *Lactobacillus rhamnosus*; MGEM, gemcitabine-loaded mesoporous silicon nanoparticles; mGN, carboxymethylated β-glucan; NE, norepinephrine; NZ9000, *Lactococcus lactis* NZ9000; PA, phenylboric acid; PEGDA, poly(ethylene glycol) dimethacrylate; PEO, polyethylene oxide; PLGA, poly-lactic-*co*-glycolic acid; PVP, polyvinyl-pyrrolidone; PTX, paclitaxel; SiH@TPGS-PEI, copolymer-modified two-dimensional H-silicene nanomaterial; SN15-2, *Bacillus subtilis* SN15-2; TA, tannic acid; YM, yeast membrane; 5-ASA, 5-aminosalicylic acid; −, not applicable.

used as a coating to protect them, and functionalized polymers can enhance the targeted therapeutic effect of probiotics and increase probiotic colonization at the target site.

To alleviate intestinal inflammation, Zhou et al.[34] engineered the oral probiotic EcN (EcN-pE) to overexpress catalase and superoxide dismutase, which are utilized to remove ROS from inflammatory areas. To enhance the bioavailability of EcN-pE in the gastrointestinal tract, EcN-pE was coated with natural polysaccharides CS and alginate (ALG) by a layer-by-layer electrostatic self-assembly strategy, and the CS/ALG-coated EcN-pE (EcN-pE$(C/A)_2$) was effective in alleviating inflammation and repairing colonic epithelial barriers in different chemically induced IBD mouse models.

Polydopamine (PDA), a natural polymer, has a variety of applications in the delivery of living cells. Due to the powerful adhesive capabilities of its catechol moiety, PDA has been widely used as a coating layer on the surface of various materials. Moreover, the polymerization reaction of dopamine can take place in cell-friendly settings. PDA can be deposited on the cell surface *via* covalent bonds (*e.g.*, Michael additions and Schiff base reactions), $\pi-\pi$ accumulations, or hydrogen bonds[66,67]. Chen et al.[67] attempted to functionalize individual yeast cells with PDA and discovered that yeast cells with PDA coating maintained their viability, which was regulated by the thickness of the shell during the cell cycle. As shown in Fig. 2B, Pan et al.[68] modified the surface of probiotics with a co-deposited coat of PDA and CS. The surface-modified probiotics improved the enrichment of the microbial community in the intestine more than the original probiotics. Furthermore, the surface coating with a protective layer and a navigational function could protect the probiotics against gastric fluid and bile acid and enhance the targeting ability. The excellent surface coating characteristics are conducive to improving the treatment of intestinal inflammation.

With the mucosal adhesion function, catecholamine-based polymers that could adhere to mucous membranes emerged in recent years. Liu et al.[33] utilized the oxidative self-polymerization of norepinephrine (NE) on the surface of probiotic EcN to form a poly norepinephrine (pNE) membrane. Then, hyaluronic acid (HA) conjugated poly(propylene sulfide) was self-assembled into HPN nanoparticles with ROS scavenging ability. These were further modified onto the NE-EcN to form HPN-NE-EcN. Based on the pNE coating helping EcN to colonize in the intestine, this system exerted an excellent therapeutic effect on IBD. Notably, the NE layer-coating strategy effectively encapsulated living EcN cells for cytoprotection, while the NE layer coating and HPN conjugation did not impact EcN's growth and proliferation. Furthermore, the HPN nanoparticles can self-degrade after fully reacting with ROS as the physicochemical transformation of PPS shifts from hydrophobic to hydrophilic, thus enhancing the safety profile of this approach.

In addition to natural polymers, the commercial polymer Eudragit plays a massive role in delivering probiotics[69]. Due to the pH-dependent properties of the polymer eutectic, pH-responsive probiotic delivery systems can be prepared. Liu et al.[70] reported a method of bilayer encapsulation involving tannic acid (TA) and Eudragit L100 to coat probiotics, aiming at overcoming the obstacles of oral drug delivery. TA and Eudragit L100 layers coated EcN showed robust resistance to the harsh external environment of

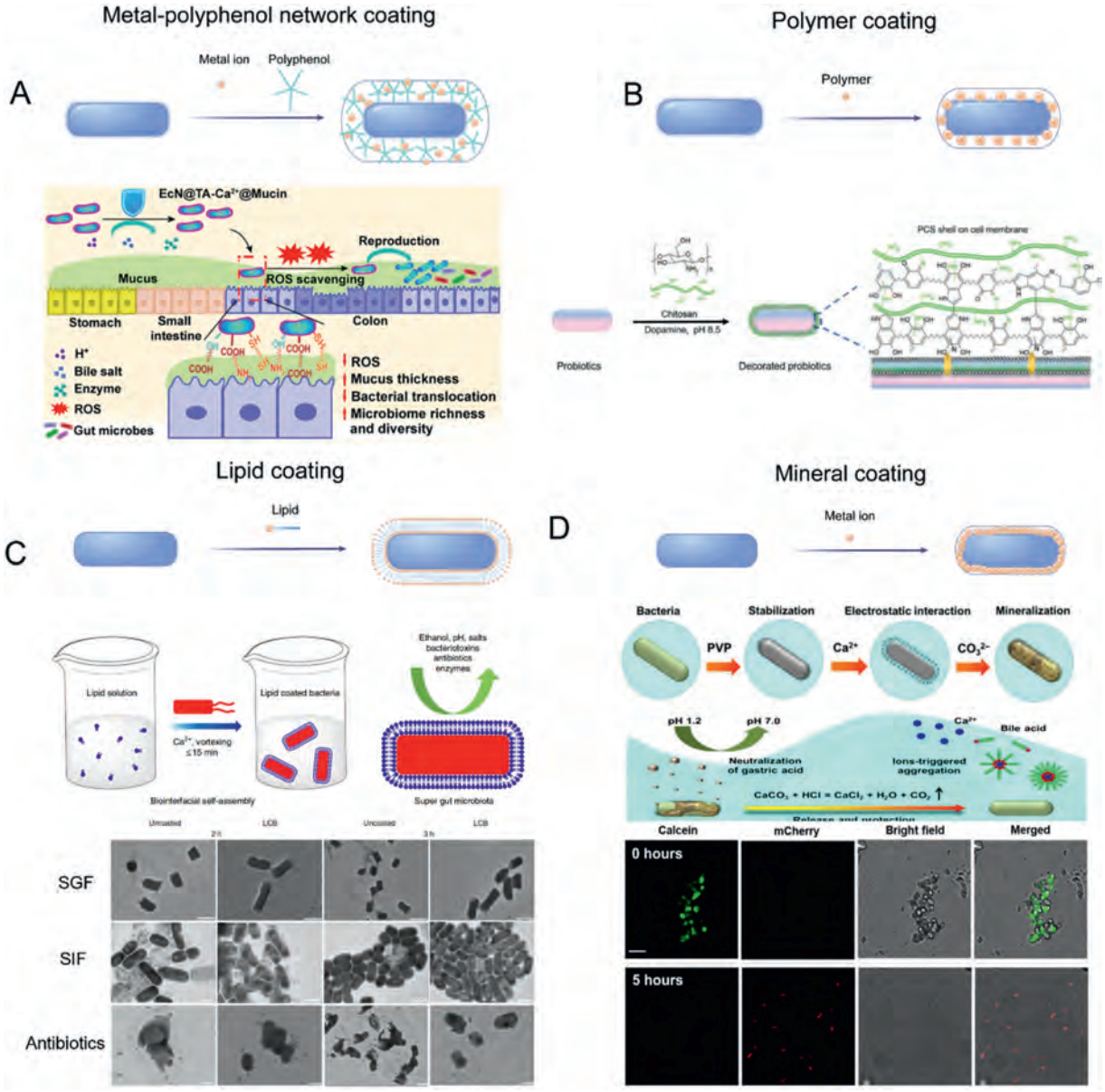

Figure 2 Metal-polyphenol network, polymer, lipid and mineral coated probiotics. (A) TA- $Ca^{2+}$and mucin-coated EcN. Reprinted with the permission from Ref. 31. Copyright © 2022, American Chemical Society. (B) The surface of probiotics was coated with a co-deposited coat of polydopamine and chitosan (CS). Reprinted with the permission from Ref. 68. Copyright © 2021, Wiley-VCH. (C) Scheme illustrating the preparation of the lipid membrane-encapsulated EcN. Reprinted with the permission from Ref. 35. Copyright © 2019, Nature Publishing Group. (D) Schematic illustration of biointerface mineralization of BF839 and confocal images of mCherry-expressing EcN coated with calcein-labeled mineral layer. Scale bar = 10 μm. Reprinted with the permission from Ref. 37. Copyright © 2023, AAAS.

the gastrointestinal tract. In addition, the pH-responsive degradation of the external Eudragit L100 layer endowed EcN with a selective intestine-targeted effect, and the strong adhesion capacity of the TA layer prolonged the retention time of EcN without affecting viability, leading to superior prophylactic and therapeutic efficacy.

Lipid coating: Apart from polymer coating, many more established packaging materials are used in pharmacies to coat probiotics. As shown in Fig. 2C, Cao et al.[35] attempted to prepare a phospholipid molecular layer as a coating material on the surface of a probiotic by self-assembly, and the presence of calcium ions condition helped the supramolecular self-assembly of dopamine hydrochloride (DOPA) on the negatively charged surface of the probiotic. Cholesterol was used to stabilize the self-assembled lipid membrane further. The raw materials used were FDA-approved phospholipid molecules (widely found in cell membranes), which were favorable for subsequent clinical trials and translation. Taking EcN, for instance, after donning a lipid membrane, the lipid membrane-coated bacteria (LCB) can withstand harsh external conditions, such as strong acids, bases, antibiotics, and alcohols with different solubilities. In another research, the same supramolecular critical self-assembly technique was employed by Zhang et al.[71] to modify LGG. Balsalazide (Bal) as the 5-ASA prodrug was planted in the phospholipid molecule layer, which chemically altered the phospholipid molecule 1-hexadecanoyl-sn-glycero-3-phosphocholine (LPC). The

lipid prodrug LPC-balsalazide (LPC-Bal) helped *lactobacillus rhamnosus* GG (LGG) resist digestion juices from the stomach and intestine to reach the colon target lesion. Once in the colon, an azo-reductase enzyme sheared the lipid layer to release the UC therapeutic drug 5-ASA. This inhibited colonic inflammation, improved the pathological environment, and created a favorable microenvironment for LGG colonization. In addition, LGG can successfully colonize the intestine and then control the intestinal microbiota to improve ulcerative colitis (UC) lesions.

Liposomes are another type of material that is used to coat probiotics in addition to lipids. Liposomes are a subgroup of lipid NPs, usually composed of phospholipids, which can form unilamellar and multilamellar vesicular structures. This enables the delivery of hydrophilic, hydrophobic, and lipophilic drugs *via* liposomes. Liposomes, which could self-assemble on the surface of probiotics, are a more substantial coating than simple lipid coatings. By self-assembling on the surface of LCB, Zhou et al.[36] used liposomes to enclose probiotics with a liposomal coat. The liposome coating had a thickness of about 100 nm, which could offer LCB good protection and increase the colonization efficiency in the intestine. In treating Parkinson's disease (PD), LCB can self-regulate the production of γ-aminobutyric acid at a constant concentration and maintain a long half-life.

In addition to oral drug delivery, the lipid-coating engineered bacteria EcN BL21 was also used for intravenous drug delivery to treat tumors. Although oncolytic bacteria have made recent preclinical strides in cancer therapy, dose-limiting toxicity has long been a problem for clinical applications. Feng et al.[72] developed a straightforward but effective technique to create charge-reversible lipid membrane-coated bacteria (CRLCB). Those bacteria could switch surface charges in neutral or acidic solutions, which leads to mild interactions with negatively charged normal cells. These characteristics of bacteria enhanced their compatibility with blood cells and the immune system. Furthermore, compared to wild-type strains, lipid-encapsulated bacteria have longer blood circulation lifetimes and lower rates of tissue capture. Therefore, engineered bacteria exhibit improved tumor specificity and anti-tumor efficacy even at low doses.

Mineral coating: Mineralization, a natural process whereby organisms create a hard exterior to protect and support soft tissues, is essential for the survival of biological systems[73]. Although oral microecological agents effectively modulate the intestinal microbiome, they are constantly subjected to multiple aggressions from the oral cavity to the intestinal tract.

As shown in Fig. 2D, inspired by the protective mechanism of mineralization, Geng et al.[37] developed an electrostatic interaction-mediated biointerfacial mineralization, which produces super-resistant and self-removable coatings on bacterial surfaces. Using the next-generation probiotic *Bacteroides fragilis* (BF839) as a model bacterium, polyvinyl−pyrrolidone (PVP) was used as a stabilizer to bind with negatively charged bacteria. Then, PVP on the bacteria surface was composited with calcium ions and sodium carbonate, respectively. The absorbed calcium ions guided the multiphase nucleation and deposition of calcium carbonate on the surface of the BF839 cells to form a mineral coating. The coating gave the bacteria strong resistance to manufacturing-related oxygen exposure, ultraviolet (UV) radiation, and 75% ethanol. After oral administration, the mineral coating could actively neutralize stomach acid and release the encapsulated bacteria through a spontaneous and rapid dual catabolic reaction. Besides neutralizing acid, the released calcium ions also promoted bile acid aggregation, allowing bacteria to survive in both gastric and bile acid environments. Further supported by the therapeutic efficacy of the coated bacteria on colitis mice, biointerfacial mineralization provides a versatile platform for developing next-generation active oral biotherapeutics. In addition, they explored the versatility of interface mineralization to coat EcN. To confirm the self-removability of the coating *in vivo*, the genetically engineered mCherry expressing EcN was coated with the calcein-labeled mineral layer and then orally administrated to mice. The luminal contents were extracted from the stomach and intestine post-administration. Notably, the calcein signal decreased, but the mCherry signal increased from 0 to 5 h, suggesting that demineralization of the coating occurred and protected the released EcN by neutralizing intragastric pH.

Other coating materials: Besides the mentioned materials, numerous other coating materials can coat probiotics using chemical or physical forces. The two-dimensional SiH@TPGS-PEI coating could protect probiotics against damage from aggressive gastric juices in an acidic gastric environment. Moreover, SiH@TPGS-PEI also decomposed naturally when it reacted with water to create hydrogen. In response to a neutral or mildly alkaline intestinal environment, probiotics release anti-inflammatory hydrogen to treat colitis[38]. Most prior attempts at surface coating probiotics usually possessed the function of protecting probiotics; however, they lacked additional therapeutic benefits. Hou et al.[74] outlined a strategy inspired by silk cocoons' potent therapeutic and protective properties that involve synergistically coating probiotic bacteria with therapeutic nano coating to enhance biotherapeutics. Silk fibroin could self-assemble onto the bacterial surface by switching from a random coil to β-sheet conformation. Thanks to its innate pharmaceutical activity and protective barrier role, silk fibroin endowed the coated probiotic with improved survival against gastric insults and enhanced therapeutic effect on colitis. Peng et al.[41] used the polysaccharide structure of alginate to chelate with calcium ions to produce a colloidal framework on probiotic EcN. At the same time, the anti-inflammatory drug 5-ASA was encapsulated in the alginate-coated EcN (EcN@Alg) to form 5-ASA loaded EcN@Alg (EcN/5-ASA@Alg). With the protection effect of alginate-based coating, the drug-loaded probiotics could resist the gastric environment. Upon arriving in the intestine, 5-ASA is released along with the disintegration of the coating. This drug delivery system protects drug-loaded probiotics from gastric juices after oral administration and enhances the therapeutic efficacy against colitis by increasing the abundance and diversity of the microbiota, alleviating inflammation and repairing the intestinal barrier.

Surface coating probiotics with various materials can enhance their stability, survivability, and therapeutic efficacy in the gastrointestinal tract. Each method has unique benefits and drawbacks. The choice of coating material should consider the specific application, the environment in which the probiotics will function, and the desired therapeutic effects. Optimizing these methods can improve the efficacy and stability of probiotic formulations, ultimately enhancing health outcomes in various conditions. The comparison of the coating methods above is listed in Table 2 as follows.

2.1.1.2. Surface conjugation on single cell. Apart from coating the whole bacteria, conjugation is another way to modify the probiotic. There are two types of conjugation methods: non-covalent conjugation and covalent conjugation[75]. Covalent conjugation and non-covalent conjugation are two distinct binding methods in chemistry. Covalent conjugation involves the

Table 2 Comparison of surface coating methods on single-cell.

Method	Advantage	Disadvantage
Metal-polyphenol network coating	1) Strong adhesion in the gastrointestinal tract. 2) Enhances probiotic stability. 3) Regulates inflammatory responses. 4) Provides a protective "armor" against antibiotics.	1) Complex synthesis and application processes. 2) Potential toxicity concerns with metal ions if not properly controlled.
Polymer coating	1) Biocompatible and easy to formulate. 2) Enhance bioavailability and stability of probiotics. 3) pH-responsive coatings enable targeted release in the intestine.	1) Time-consuming layer-by-layer techniques requiring careful optimization. 2) Synthetic polymers raise concerns about biocompatibility and biodegradability.
Lipid coating	1) Shield probiotics from acids and bile. 2) Capable of encapsulating both hydrophilic and hydrophobic compounds. 3) Enhance compatibility with bodily fluids using the charge-reversible lipid membranes.	1) Complicated manufacturing processes 2) Increased costs. 3) Potential instability during storage
Mineral coating	1) Mimics natural mineralization processes. 2) Enhances survivability in acidic and bile-rich environments. 3) Effective probiotic release with self-removable coatings.	1) Specific conditions that may limit scalability. 2) Difficulties in controlling the thickness and uniformity of the coating.
Other coating materials	1) Silk fibroin offer inherent pharmaceutical activity and protection. 2) Alginate effectively encapsulates therapeutic agents and protects probiotics in the gastric environment. 3) Some coatings provide additional therapeutic benefits.	1) Complex and time-consuming 2) Therapeutic efficacy depending on the specific material and method.

formation of a chemical bond between two or more atoms through the sharing of electron pairs. On the other hand, non-covalent conjugation refers to weak interactions between atoms or molecules, such as electrostatic forces, van der Waals forces, or hydrogen bonds. Non-covalent conjugation methods include electrostatic interactions, *in situ* deposition, and lipid-insertion methods, which are simple and easy to operate on the surface of probiotics. Nonetheless, covalent conjugation methods, such as metabolic labeling and click reaction, are more stable than non-covalent conjugation. Surface conjugation of probiotics is a simple yet efficient strategy, which not only gives probiotics the extra ability to resist external environmental threats but also endows them with exogenous qualities that are neither innate nor naturally attainable in a controlled manner[76].

Non-covalent conjugation: Various lipid nanoparticles are frequently used to modify probiotics with organic substances. For instance, Akolpoglu et al.[77] combined magnetic nanoparticles with nanoliposomes (NLs) containing photothermal agents and chemotherapeutic molecules to create ICG-DOX NLs on EcN, with an integration efficiency of about 90%. As shown in Fig. 3A, this biohybrid microrobot was constructed by attaching the synthetic materials, streptavidin magnetic materials and nanoparticles (mNPs), and biotinylated NLs to EcN *via* non-covalent interactions. Compared with the control group, the bacterial biohybrids, when applied to cancer cells in a 3D tumor sphere model, led to a high mortality rate, which was further elevated under NIR radiation. The bacterial biohybrids outperformed the previously described EcN-based micro-robots because they retained their original motility, which could move through biological matrices and colonize in tumor spheroids when exposed to magnetic fields. They could also release drug molecules on demand in response to near-infrared light stimulation. These bacterial biohybrids provided a versatile micro-robotic platform for guided locomotion in three-dimensional biological networks and provided stimulus-responsive therapies for a variety of medical applications.

Additionally, paclitaxel (PTX) and the glucose transporter 1 (GLUT1) inhibitor BAY-876 were added to human serum albumin to create a nanomedicine (HPB), which was then electrostatically grafted onto the probiotic EcN to create EcN@HPB. Due to the targeted effect of EcN, this engineered biohybrid significantly increased the ability of HPB nanomedicine in the tumor site. The released BAY-876 inhibitor subsequently prevented the internalization of glucose by tumor cells after EcN competitively depleted glucose. In summary, the decreased glucose bioavailability in tumor cells activated and promoted macropinocytosis in an adenosine monophosphate (AMP)-activated protein kinase (AMPK)-dependent manner, increasing cellular HPB uptake and improving the therapeutic effectiveness of PTX[43].

Metallic materials such as metal−organic frameworks (MOFs) are typically used to modify probiotics. As shown in Fig. 3B, Han et al.[44] created E@Fe-DOX by depositing adriamycin (DOX) loaded iron-polyphenol (Fe-DOX) nanoparticles on *E. hallii*, which can be specifically targeted to hypoxic tumor regions, to release both DOX and Fe^{3+} for immunogenic cell death (ICD). The mapping images of C, N, and Fe elements indicated that Fe-DOX nanoparticles were located on the surface of the probiotic. Lactate is abundantly expressed in the tumor microenvironment (TME), and *E. hallii* could change lactate into butyrate. *E. hallii* prevented the polarization of pre-tumor M2-like macrophages and enhanced the activity of cytotoxic T cells by continuously converting intratumoral lactate to butyrate. Subsequently, E@Fe-DOX promoted the development of tertiary lymphoid structures (TLS), a site of immune cell aggregation to boost ICD-induced anti-tumor immunity. In the colon cancer mice model, the combination therapy of E@Fe-DOX and α-PD-1showed a prolonged mice survival rate compared to the PBS group.

Wei et al.[78] constructed resiquimod (R848) loaded poly-lactic-*co*-glycolic acid (PLGA) nanoparticles (defined as PR848) and DOX-loaded PLGA nanoparticles (defined as PDOX) by using the emulsification solvent evaporation method, respectively. Then,

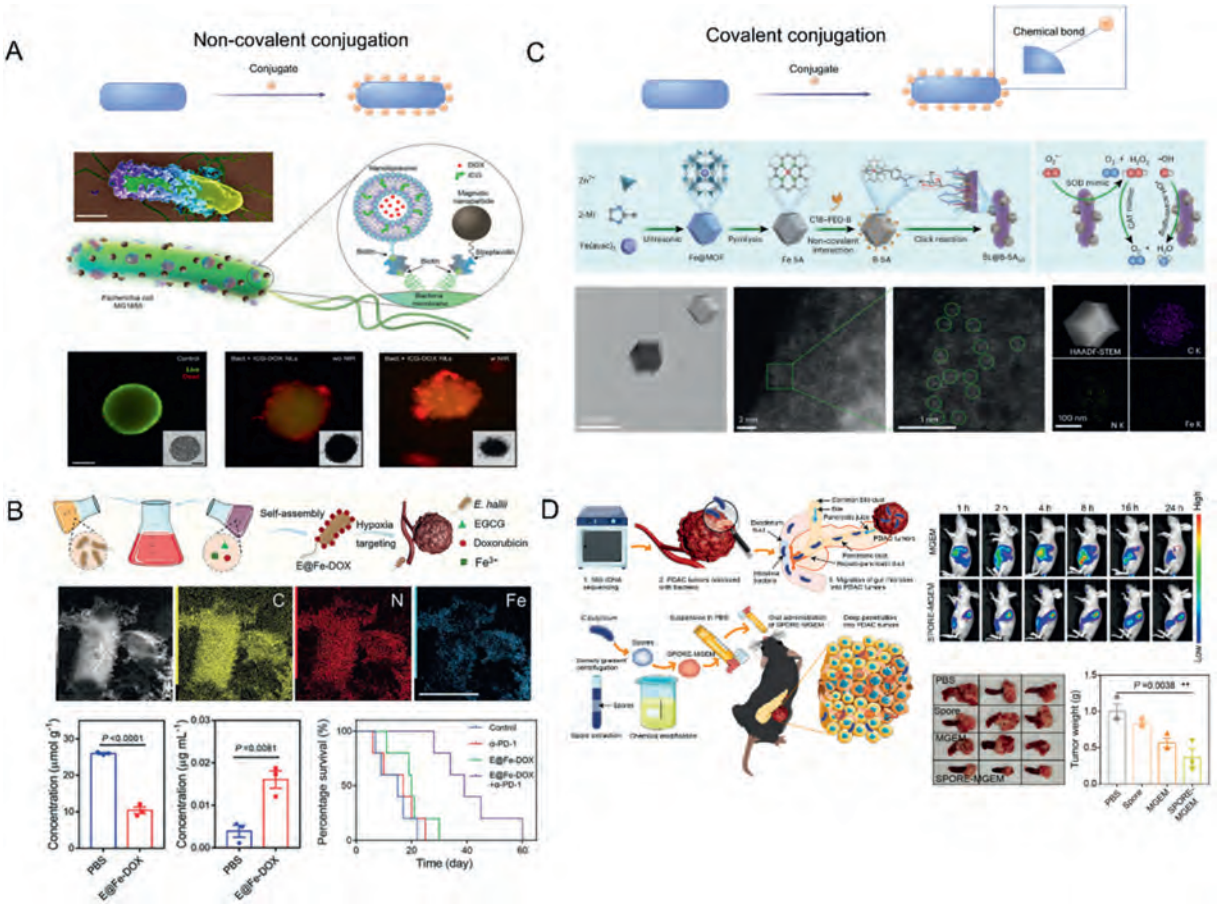

Figure 3 Non-covalent and covalent conjugation of probiotics. (A) The construction of bacterial biohybrids carrying mNPs and NLs and the cancer cell killing efficiency. Reprinted with the permission from Ref. 77. Copyright © 2022, AAAS. (B) The construction, element mapping and treatment efficiency of E@Fe-DOX. Reprinted with the permission from Ref. 44. Copyright © 2023, Wiley-VCH. (C) The construction of BL@B-SA50. Reprinted with the permission from Ref. 46. Copyright © 2023, Springer Nature. (D) The construction, whole-body distribution and treatment efficiency of biohybrid system SPORE-MGEM. Reprinted with the permission from Ref. 45. Copyright © 2022, American Chemical Society.

electrostatically, PR848 nanoparticles were linked to non-pathogenic EcN MG1655 to form Ec-PR848. After intravenous injection (i.v.), Ec-PR848 could target hypoxic tumors and polarize M2 macrophages to M1 macrophages to stimulate the immune system's anti-tumor responses. Meanwhile, the intratumorally injected PDOX triggered ICD to encourage dendritic cells (DCs) maturation as well as antigen presentation and to activate cytotoxic T lymphocytes (CTLs) for the weakening of tumor immunosuppression, which in turn activated the anti-tumor immune response and killed tumor cells.

Covalent conjugation: Cao et al.[46] reported an artificially enzyme-modified platform based on the probiotic *Bifidobacterium longum* (BL) to treat IBD (Fig. 3C). This platform used an iron single-atom catalyst (Fe SA) nanomaterial with atomically dispersed active metal centers to mimic the natural antioxidant defense system effectively and replace anti-inflammatory clinical medications. Then, boronic acid−poly(ethylene glycol) (C18-PEG-B)-modified Fe SA (defined as B-SA) was conjugated onto BL *via* click reaction to obtain the probiotics-artificial enzymes system of BL@B-SA50. This single-atom catalyst artificial enzymes can imitate the antioxidant enzymatic activities of superoxide dismutase (SOD) and catalase (CAT) to scavenge superoxide radicals (O_2^-) and hydrogen peroxide (H_2O_2) and act as antioxidant biomolecules to scavenge hydroxyl radicals (−OH), thereby delivering a potent and quick relief of inflammatory symptoms. Additionally, this synthetic enzyme may serve as a probiotic guardian to shield probiotics in capsules from oxidative damage due to high levels of ROS in inflamed tissues and quickly restore intestinal barrier function and the balance of the intestinal microecosystem.

Due to insufficient intratumoral delivery of antitumor agents, the chemotherapy of pancreatic ductal adenocarcinoma (PDAC) is severely hindered. To alleviate this problem, Han et al.[45] employed an improved chemotherapy for pancreatic cancer (Fig. 3D). More chemotherapeutic drugs were delivered to the tumor site *via* the gut−pancreas axis by using a probiotic spore-based oral medication delivery method. Commercial *Clostridium butyricum* (*C. butyricum*) spores were covalently grafted with the powerful cytotoxic drug gemcitabine-loaded mesoporous silica nanoparticles (MGEM). The result was that hybridized SPORE-MGEM significantly increased intra-tumor drug accumulation and improved antitumor efficacy *via* gut-pancreas axis-guided delivery.

2.1.2. *Multiple cells encapsulation*

Single-cell probiotics are modified primarily for surface coating, whereas multiple-cell probiotics are modified for bulk encapsulation to protect probiotic cells. Typically, the encapsulated probiotics are given orally or *in situ*. However, before the probiotics enter the target organ, they should navigate a complex

environment that includes digestive fluid and a variety of enzymes[79]. Therefore, selecting coating materials is necessary. Natural polysaccharides, including CS[80], ALG[81,82], and HA[83], have recently become widely employed. These natural and biocompatible materials can be used as coating components to encapsulate probiotics and form diverse dosage forms.

2.1.2.1. Hydrogel. The biocompatible hydrogels with a unique 3D cross-linked network structure allow them to hold large amounts of water and bodily fluids to avoid disintegration. Usually, one or more organic or inorganic polymers link together through covalent bonds or non-covalent bonds (*e.g.*, electrostatic interactions, hydrophobic interactions, and hydrogen bonds) to form the hydrogels. Polysaccharides (*e.g.*, ALG, CS, HA) and protein (gelatin and whey protein, etc.) are the most frequently used structural components of hydrogel matrix, respectively as physical barrier and buffer, which endow hydrogel with the expansion, water-absorbing quality and pressure tolerance. The existing oral hydrogels often take advantage of differences in pH, chemical composition (*e.g.*, stomach acid, bile salts), or digestive enzymes between the stomach and intestinal to pre-determine the timing of the disruption of probiotic−hydrogel interaction, allowing hydrogels to pass through the stomach and deliver probiotics to intestinal[84].

Yang et al.[85] created an oral probiotic delivery system based on calcium tungstate microgel (CTM) by cross-linking calcium ions with ALG. In Fig. 4A, CTM deliberately disrupted the ecological niche of the excessively expanded Enterobacteriaceae in colitis to promote probiotic colonization. Additionally, the calprotectin, as the calcium-binding protein that is abundantly expressed in colitis, efficiently removed calcium from CTM to release tungsten, which dislodged molybdenum in the molybdenum enzyme to inhibit Enterobacteriaceae. For the encapsulation and targeted distribution of probiotics, a ROS-responsive HA hydrogel was created by Huang et al.[86] The methacrylated HA and thioredoxin were cross-linked biologically to obtain HA-LR. LR, a model probiotic encapsulated in the hydrogel, showed a significantly better survival rate under simulated digestive conditions. The negatively charged hydrogel helped probiotics preferentially adhere to inflammatory areas. The thioredoxin link in the hydrogel was selectively broken by excess ROS produced by inflamed colonic mucosa, accompanied by hydrogel disintegration and localized probiotic release. Additionally, the hydrogel had a sufficient capacity to scavenge ROS and shield HT-29 cells from oxidative injury.

Besides treating digestive diseases, probiotics are often crucial in treating different illnesses. One example is the introduction of probiotics for the treatment of vaginitis. Although molecular therapeutics are frequently used to treat *Candida* vaginitis, the high recurrence rate hindered their application, and these methods destroy vaginal tissues and exacerbate the vaginal microbiota's imbalance. In Fig. 4B, Wei et al.[87] combined the rGO@FeS_2 nano-enzymes [reduced graphene oxide (rGO)] with *Lactobacillus* (a probiotic that generates lactic acid and H_2O_2) to create a responsive hydrogel-rGO@FeS_2/*Lactobacillus*@HA (FeLab). FeLab significantly reduced bacterial growth in mice with *Candida* vaginitis while causing bare harm to vaginal mucosal cells and encouraged the healing of the mucosa. Additionally, with an upsurge in the proportion of the thick-walled bacterial phylum (particularly *Lactobacillus*) and a decline in *Aspergillus*, the vaginal microbiota was remodeled, lessening the likelihood of inflammation returning.

2.1.2.2. Microspheres and microcapsules. Apart from the probiotic-based hydrogels, hydrogels can also form microspheres. Probiotics or functional food molecules are encapsulated in

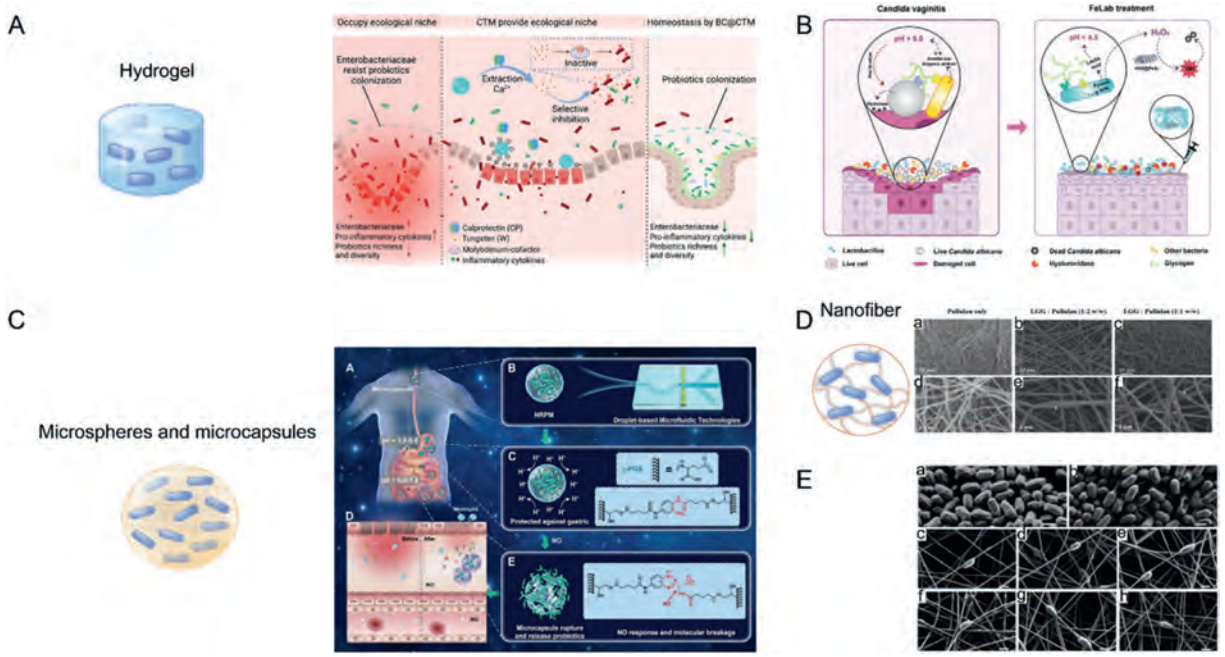

Figure 4 Hydrogel, microspheres and microcapsules, and nanofiber encapsulated probiotics. (A) The construction of an oral probiotic delivery system based on CTM. Reprinted with the permission from Ref. 85. Copyright © 2023, American Chemical Society. (B) The construction of a responsive hydrogel FeLab. Reprinted with the permission from Ref. 87. Copyright © 2023, AAAS. (C) Schematic illustration of intestinal-targeted NRPM using a pH-responsive gel microsphere. Reprinted with the permission from Ref. 88. Copyright © 2022, Wiley-VCH. (D) Electrostatic spinning is used to create a nanofiber to shield the probiotic LGG. Reprinted with the permission from Ref. 57. Copyright © 2022, Elsevier. (E) The SEM of PEO and composite PEO/ALG nanofibers. Scale bar = 1 μm. Reprinted with the permission from Ref. 58. Copyright © 2023, Elsevier.

hydrogel microspheres, a unique emulsion-based delivery medium with good biocompatibility and controlled release features. The probiotic-based hydrogel microsphere therapy is efficient and secure for treating intestinal illnesses. As shown in Fig. 4C, Wang et al.[88] formed a novel nitric oxide (NO)-responsive Poly-γ-glutamic acid hydrogel microcapsules (NRPM) to encapsulate *Lactobacillus*. Microspheres with wide-ranging diameters (100−600 μm), high cell densities, and homogeneity (6.0×10^8 cells/mL) were required to allow accurate downstream evaluation and application. The modified probiotics displayed high survivability in simulated gastric (89.67%) and intestinal (93.67%) fluid environments because of the cytoprotective impact of NRPM, whereas the data of free cells were 0% and 61.60%, respectively. Additionally, *in vitro* and *in vivo* research suggested that microspheres responded to NO stimulation and released probiotics fleetly, maintaining the intestinal barrier and regulating the intestinal flora balance.

In the study of Cheng et al.[89], layer-by-layer electrostatic droplet self-assembly of alginate and protamine was used to create enzyme-triggered fusion-like microcapsules. This study demonstrated that the probiotics could be shielded from acid and bile acid by the fusion structure of two fisetin layers in alginate microcapsules, which could then be broken down layer by layer by trypsin to regulate bacterial release, aid in bacterial adhesion, and effectively colonize the proximal colon and distal ileum.

2.1.2.3. Nanofiber. In 2006, Salalha et al.[90] attempted to encapsulate bacteria (*Escherichia coli*, *Staphylococcus albus*) in electrospun nanofibers. The most widely used technique for creating nanofibers is electrospinning[70]. Electrospun nanofibers have a high specific surface area and porosity, which can preserve the bioactivity of the encapsulated compounds and facilitate the release of bioactive molecules.

Nanofiber-encapsulated probiotics have attracted more attention recently[91]. Ajalloueian et al.[57] created a nanofiber to shield the probiotic LGG using electrospinning technology. As shown in Fig. 4D, Pullulan, positioned between the inner and outer layers of PLGA, protected the three-layer electrostatically spun nanofiber. LGG delivered by the multilayer structure could survive intestinal transport and recover activity in all areas of the intestine.

In Fig. 4E, Grilc et al.[58] incorporated spores from two different *Bacillus* strains, individually or in combination, into hydrophilic polyethylene oxide (PEO) and composite PEO/ALG nanofibers. The nanofiber mats had a significant load of viable spores (>7 log CFU/mg), and the vitality of the spores was maintained throughout electrospinning after six months of storage at room temperature. Spores were rapidly expelled from PEO nanofibers, but the ALG in them prolonged their release. The produced nanofiber contained genotyped novel prospective probiotic strains with simultaneous immunomodulatory and antibacterial activities that were demonstrated to be a promising tool for local therapy of biofilm-associated illnesses, including periodontitis[58].

Treatment for microbiological disruptions of the female genitourinary system often involves the usage of antibiotics. Probiotics are increasingly being used as an adjuvant and alternative to antibiotic therapy, which calls for the development of innovative delivery methods for vaginal use. Minooei et al.[92] developed a novel, quick-dissolving platform that employed electrospun PEO fibers to deliver *Lactobacillus acidophilus* (LAC) and the antibiotic metronidazole. The vaginal colonization of probiotics encapsulated in PEO fibers achieved the same level as free LAC.

Conventional physical modification strategies are generally straightforward and safe, largely because they do not require additional toxic chemical agents. Multiple cells encapsulation, which can protect the probiotics from the harsh gastrointestinal environments, often realizes the bulk encapsulation of probiotics[93]. However, this approach has some limitations, including low encapsulated efficiency, early release of probiotics, and a time-consuming process.

Compared with the conventional multiple cells encapsulation, the single-cell coating can overcome the issues for probiotic delivery and provides some unique features, such as resistance to the *in vivo* gastric environment, improved colonization, and a potential therapeutic effect in treating related diseases. However, the single-cell coating of probiotics still has some drawbacks. For instance, the layer-by-layer self-assembly technique is cumbersome and time-consuming, which hinders its large-scale application[94]. Overall, enhancing the coating efficiency of probiotics and increasing the resistance of coatings to gastric juice and bile acids will undoubtedly promote the biomedical use of probiotics.

Therefore, chemically modifying probiotics is an effective approach for single-cell modification, which can protect them from harsh environments, thereby enhancing their survival rate. Additionally, chemical modification can increase the colonization rate of probiotics in the intestine, extend their intestinal retention time, and improve treatment outcomes for related diseases. Controlled release of probiotics can also be achieved after chemical modification by incorporating stimuli−responsive properties. Furthermore, chemical conjugation can introduce additional functional substances to probiotics, endowing them with enhanced functions to improve therapeutic efficacy and broaden their applications. However, there is still room for improvement in chemical engineering strategies, such as developing more biocompatible materials and mild reaction conditions that do not compromise bacterial cell viability and reducing the use of chemical reagents.

2.2. *Biomedical modification*

Synthetic biology and microbiology advancements have led to significant progress in creating genetically modified probiotics for various biomedical purposes. Biological engineering methods are preferred over physicochemical modifications because they are more compatible and secure for probiotics due to their limited use of external chemicals and covalent bonds. While physicochemical techniques can modify a wide range of bacteria by targeting common functional groups on their surfaces and giving them various external properties, biological engineering techniques can introduce lasting functions[95].

2.2.1. *Genetic engineering*

Traditional methods make it challenging to generate enough therapeutically active proteins and peptides required to treat endocrine and cancerous illnesses, which restricts their widespread application. The unique characteristics of probiotics, such as safety, lesion targeting, tissue retention ability, gene editing, and high proliferation rates, have shown significant application potential in treatment, biological imaging, and diagnosis[96,97]. Due to the rapid development of synthetic biology, researchers are now focusing on engineering probiotics as bioactive factories to express various therapeutic components *in situ*. It is worth noting that various types of recombinant proteins have been effectively expressed since the development of engineering bacteria. For example, the first recombinant protein product—human insulin, was introduced in 1982, sparking researchers' enthusiasm for using genetic engineering to create recombinant protein

therapies[98]. So far, numerous recombinant protein medications have already hit the market, which has had significant positive economic and societal effects.

As shown in Fig. 5A, phenylketonuria is a metabolic disorder characterized by high levels of phenylalanine (Phe) in the blood. Isabella et al.[99] genetically modified EcN 1917 to overexpress Phe metabolic enzymes, significantly reducing circulating Phe during intestinal administration. Probiotics are genetically modified to express a range of antibodies to elicit an immune response and defend against particular bacterial or viral infections. For instance, in Fig. 5B, Chowdhury et al.[21] engineered a non-pathogenic *Escherichia coli* strain to specifically lyse within the tumor microenvironment and release an encoded nanobody antagonist of CD47 (CD47nb), which further increased the activation of tumor-infiltrating T cells and stimulated rapid tumor regression in a syngeneic tumor model in mice. Additionally, there is growing evidence linking IBD to intestinal microbiome[22,23]. Engineering probiotics have been utilized to provide anti-inflammatory chemicals in treating Crohn's disease (CD), UC, and IBD. As shown in Fig. 5C, Praveschotinunt et al.[100] genetically modified EcN to create fibrous matrices that promote gut epithelial integrity *in situ*. In addition to EcN, many other probiotics are also used for genetic engineering, such as *Lactobacillus*, which was engineered to continuously deliver glucagon-like peptide-1 (GLP-1) in the intestine to treat memory disorders induced by lipopolysaccharide (LPS) and improve spatial learning in mice[59]. In addition, *L. lactis* was edited to produce anti-inflammatory cytokine interleukin-10 (IL-10), which can effectively relieve colitis symptoms in mice[60]. By converting *Lactobacillus casei* into a toxoid-toxin that expresses and secretes *Clostridium perfringens*, Gao et al.[61] created a vaccination against *Clostridium perfringens* infection, providing a more theoretical and practical basis for expanding the application of engineered probiotics.

Genetically engineered probiotics have great potential as highly targeted, self-sustaining therapeutic agents that adapt and respond dynamically to the body's needs, creating *in situ* therapeutic responses based on specific biological signals. For instance, these engineered probiotics could be designed to sense particular biomarkers associated with disease states, such as inflammation in the case of IBD, and respond by producing tailored therapeutic molecules (like anti-inflammatory cytokines) only when needed, enhancing both efficacy and safety. Additionally, combining genetically engineered probiotics with immune-modulating functionalities presents a promising area for cancer immunotherapy, as probiotics could be engineered to enhance immune activity directly within tumor sites, which could personalize cancer treatment while minimizing systemic side effects.

2.2.2. *Biofilm encapsulation*

Microorganisms are often surrounded by a thick, dense extracellular polymeric matrix that they secrete, primarily composed of polysaccharides, proteins, and extracellular DNA. This complex structure, known as a biofilm, enables microorganisms to thrive in extreme conditions and resist various physical and chemical threats, including environmental removal, displacement by physical forces, exposure to antibiotics, and attacks by the host immune system. Inspired by the robust resistance of biofilms to harsh environments, probiotics that can form biofilms or are coated with biofilms gain improved adhesion and resilience in transplanted gut microbiota. There are two categories of biofilm origins: biofilm sourced from other organisms and biofilm generated by probiotics.

The YM and EcN were physically extruded through a porous polycarbonate membrane with a diameter of about 1 μm to create YM-encapsulated EcN (EcN@YM). Due to the high expression of β-glucan on YM, the Dectin-1 receptor on the microfilmed cells (M-cells) in the intestinal epithelium may selectively attach to it

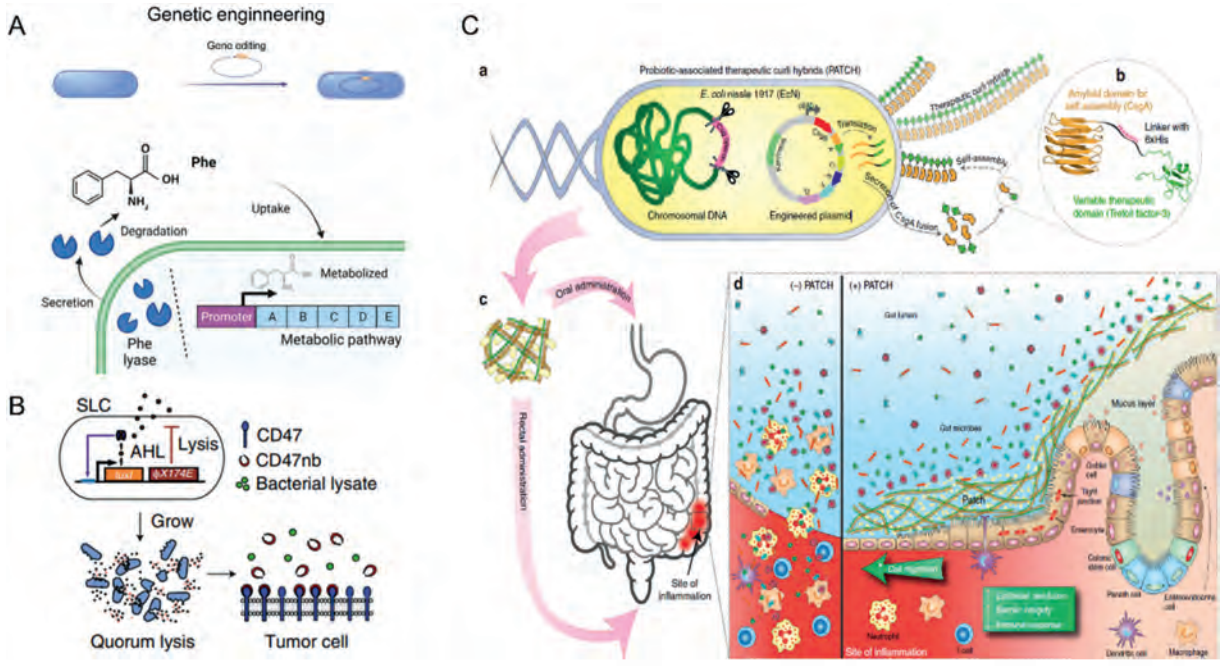

Figure 5 Engineering probiotics as bioactive factories. (A) Engineered EcN to express genes encoding Phe-metabolizing enzymes. Reprinted with the permission from Ref. 99. Copyright © 2022, AAAS. (B) Engineered EcN is constitutively expressing CD47 nanobody to induce anti-tumor immunity. Reprinted with the permission from Ref. 21. Copyright © 2022, AAAS. (C) Engineered EcN for IBD treatment by curli production *via* self-assembly of chimeric CsgA proteins. Reprinted with the permission from Ref. 100. Copyright © 2019, Springer Nature.

and internalize EcN@YM after oral delivery. M-cells transferred the absorbed live bacteria to lymphoid follicles, which could strengthen the mucosal immune response. In the gut, there were noticeably high levels of secretory immunoglobulin A (sIgA), $CD11c^+$ DCs, $CD4^+$ T-cells, and IgA^+ B-cells. In particular, pathogens such as *Salmonella*, EcN, and *Shigella* were largely repressed, while commensal bacteria were successfully kept in the infected gut due to the improved immunity[62]. Finally, this yeast membrane-camouflaged probiotic substantially reduced intestinal barrier disruption in the *Salmonella*-infected mouse and intestinal manipulation models.

Other academics have been interested in the possibility of using probiotic spores as a natural biofilm to protect probiotics in addition to using yeast membrane biomimicry. As shown in Fig. 6A, by *in vitro* mechanical extrusion, Song et al.[101] created nanomaterials from the probiotic spore shell, which then acted as "armor" to encapsulate probiotics. In addition to enhancing the natural biological activity and intestinal colonization capacity of probiotics, probiotics enclosed in spore coat nanomaterials could maintain the homeostasis of intestinal commensal flora and repair the integrity of the intestinal mucosal barrier. The probiotics were able to reduce the symptoms of colitis greatly and showed success in preventing colitis-related cancers.

In Fig. 6B, Wang et al.[102] developed a self-coating strategy with biofilms produced by probiotics themselves. *Bacillus subtilis* (BS) can generate enormous amounts of extracellular polysaccharides and proteins such as TasA and BslA, creating a biofilm when BS grows on glycerol glutamate (Msgg) plates. Polysaccharides connect the probiotics, BslA creates a hydrophobic membrane, and TasA self-assembles into fibers, adhering to the cell wall. For the production of solid biofilms, the minimal salts Msgg culture plate serves as an attachment site. The membrane was homogenized to create individual biofilm-encapsulated BS (BCBS). Encapsulated BCBS showed a 125-fold increase in biological activity and a 17-fold increase in colonization capability compared to unencapsulated BCBS *in vivo* investigations on a pig model. BCBS also showed an increased decolonization effect in a mouse model of *Staphylococcus aureus* (SA) infection.

Similarly, incubation with biocompatible dextran microspheres (DM) could stimulate the biofilm produced by *Lr*. Additionally, to promote the creation of biofilms, these microspheres might be filled with advantageous ingredients such as sucrose or maltose. As a preventative measure and a form of therapy, a single dose of *Lr* in its biofilm stage lessens the severity and frequency of experimental *C. difficile* infections[63].

Despite significant advancements in probiotic modification, several challenges remain unresolved. First, physically modified probiotics suffer from low encapsulation efficiency and premature release[103]. Second, chemically modified probiotics face high costs, environmental concerns, potential toxicity to probiotics, and risks to human health. For genetically engineered probiotics, limited genetic engineering platforms and incomplete genomic

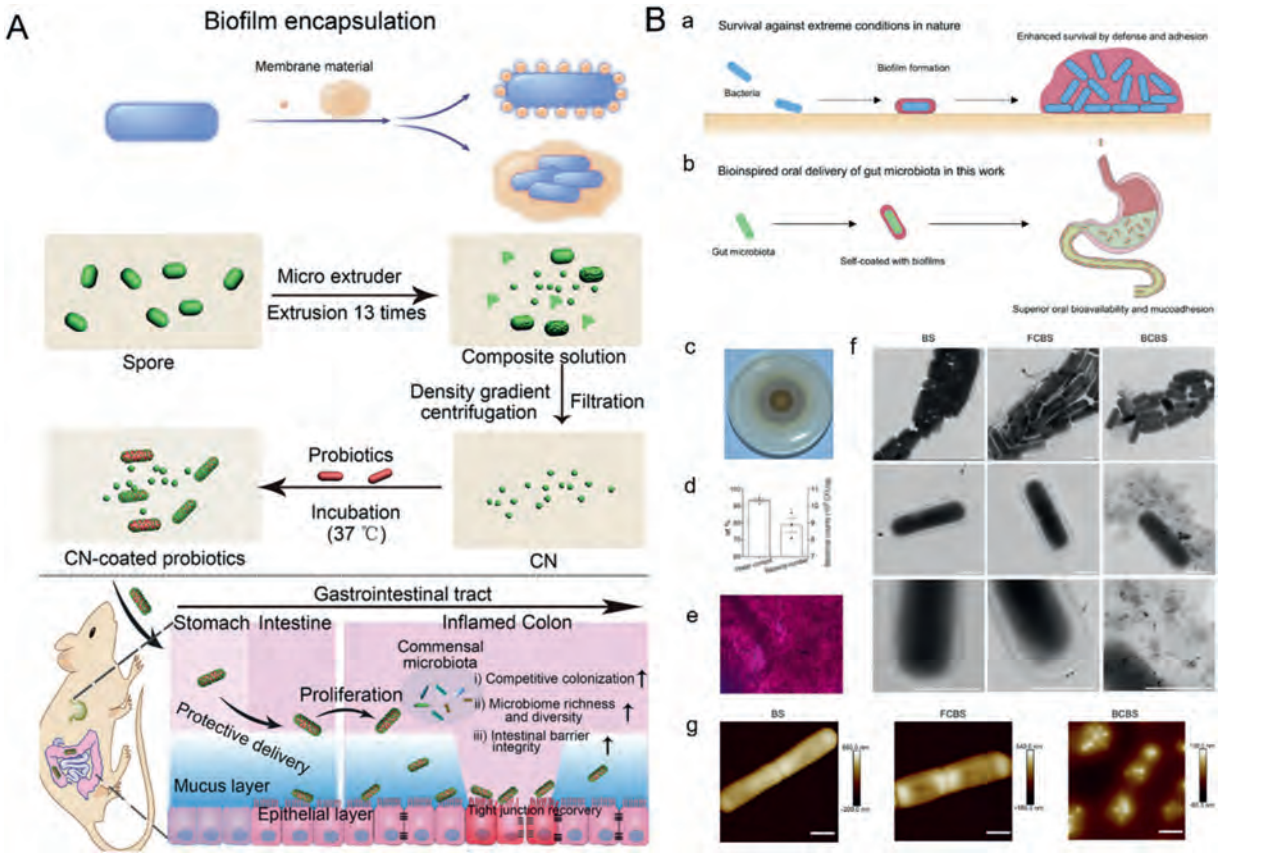

Figure 6 Biofilm encapsulated probiotic. (A) Schematic illustration of the preparation of CN-coated probiotics, and the mechanism for treating colitis. Reprinted with the permission from Ref. 101. Copyright © 2021, Wiley-VCH. (B) Design of biofilm self-coating strategy for the delivery of BS and characterization of the probiotic after biofilm self-coating. Scale bar = 1 μm. Reprinted with the permission from Ref. 102. Copyright © 2020, AAAS.

information for some strains pose challenges to their broader application. To overcome these issues, new materials should be explored for probiotic modification, such as probiotic spores, fungal cell walls, microalgae shells, and other advanced artificial approaches like microfluidics and bioprinting. Furthermore, it is crucial to gather more genomic information on other probiotics and develop efficient and reliable genetic manipulation techniques for different probiotic species to obtain diverse functional probiotics with enhanced intestinal delivery efficiency.

3. Modified probiotics in combinatorial therapeutics

Probiotic therapies have shown great potential in disease treatment. However, some disorders, like severe diarrhea, inflammatory bowel disease, and colon cancer, cannot be treated with the use of probiotics alone. The characteristics of probiotics, including rapid growth ability, anaerobic tendency, and immunomodulatory function, make them suitable as drug delivery vehicles. As a result, probiotic-based drug delivery has attracted much attention from scholars. Furthermore, combining probiotics with other therapies can lessen single therapies' adverse effects while enhancing and amplifying the therapeutic effectiveness. Probiotic-based combination therapy offers a new course of treatment for gastrointestinal diseases or other diseases that benefit from probiotic therapy[104].

3.1. Combination of modified probiotics and pharmacotherapy

To achieve optimal therapeutic effects, the majority of anti-IBD drugs must reach the colon at high concentrations. However, 5-ASA and its prodrugs are easily absorbed and eliminated in the upper gastrointestinal tract following oral administration, implying that drug accumulation in the colon is restricted[105]. Moreover, most nanomedicines cannot penetrate the dense tumor mesenchyme due to the disordered vascular system and high tumor mesenchymal pressure, and only a few nanomedicines reach the tumor site[106-109]. Therefore, improving the effect of drug targeting and bioavailability remains a significant challenge. Probiotic therapies show enormous promise in improving the combined efficacy. Combining probiotics with drugs or drug-loaded nanoparticles has demonstrated outstanding anti-tumor efficacy[110,111].

The complex and variable gastrointestinal microenvironment leads to suboptimal colonization efficiency of most probiotics, limiting their wide application in disease treatment[112,113]. Zhang et al.[71] assembled the anti-UC prodrug Bal onto liposomal LPC to form a prodrug nanomolecule LPC-Bal to enhance probiotic colonization while maintaining bacterial activity. Then, LPC-Bal was modified onto the surface of LGG to obtain a prodrug-encapsulated LGG, abbreviated as LBL. The LBL effectively increased the colonization efficiency of LGG, improved the pathological microenvironment, reshaped the gut microbiota composition, and exhibited outstanding anti-UC properties (Fig. 7A). In addition, several studies have shown that both parthenogenetic anaerobic bacteria, such as *Clostridium difficile*, and probiotics, such as Bi and EcN, possess the intrinsic tumor-targeted ability due to their instinctive tendency towards hypoxic and eutrophic tumor microenvironment. Therefore, precise delivery of drugs to the tumor region can be achieved by modifying drug-carrying nanocarriers on the bacterial surface. The Bi was covalently modified with DOX-loaded CaP/SiO2 nanoparticles (DNPs) to construct the DNPs@Bi for tumor targeting therapy by He et al. (Fig. 7B). On the one hand, pH-responsive DOX release could induce chemotherapy and immunogenic death in the immunosuppressive tumor microenvironment (ITME). On the other hand, tumor-targeted Bi was able to significantly enhance tumor-associated antigen presentation from B16F10 cells to DCs *via* recombinant connexin 43 (Cx43)-dependent gap junctions[114].

Controlled release of drugs within the target site is challenging to achieve, particularly in fluctuating microenvironments like tumors or inflamed tissue. However, researchers have identified probiotic spore (PS) as a potential carrier for controlled drug release. PS is a dormant life form with low water content and high resistance to harsh environments such as high temperatures, acids and alkalis, ultraviolet rays, and a variety of chemicals[115]. PS can resist extremely harsh gastric environments. Moreover, after reaching the intestine, PS absorbs water and sheds hydrophobic spore coat proteins to regerminate into probiotics and colonize the intestine[116,117]. After colonization, *Bacillus* initiates multiple biological actions, such as regulating intestinal flora, secreting therapeutic metabolites (*e.g.*, SCFAs), and enhancing innate and adapted immunity[118]. Inspired by this natural process, researchers have organically combined probiotic spores with drugs or drug-loaded nanoparticles through hydrophobic interaction, electrostatic adsorption, hydrogen bonding, etc. This method significantly achieves the natural-spore-germination-based drug controlled release profile and improved drug bioavailability. As shown in Fig. 7C, Song et al.[119] developed an oral nanoparticle generator where the spores were loaded with chemotherapy drugs (doxorubicin, sorafenib, DOX/SOR) and modified with deoxycholic acid (DA). On the one hand, the nanoparticle generator protected the loaded drugs from the harsh intestinal environment. On the other hand, the autonomously generated DOX/SOR/Spore-DA nanoparticles could effectively penetrate epithelial cells, thereby increasing drug release efficiency on the basal outer side. The *in vitro* and *in vivo* studies have shown that the nanogenerator autonomously generated many nanoparticles in the intestine, providing a new strategy for cancer treatment. In Fig. 7D, Zheng et al.[120] chemically modified prebiotic Dextran (Dex) onto the commercial *C. butyricum* spores to obtain Spores-Dex through a host-guest chemical reaction. The results indicated that Spores-Dex could specifically accumulate in tumor sites after oral administration. Dextran can be fermented by bacteria such as *C. butyricum* and produce short-chain fatty acids, significantly increasing the overall richness of the microbiota. This work reveals the possibility of using highly safe strategies to regulate gut microbiota and provides promising avenues for treating various gastrointestinal diseases. In addition, probiotics/spores and drugs/drug-loaded nanoparticles can also be encapsulated by hydrogels or microspheres to promote the colonization of probiotics and control the release of drugs through conditional responsive release[41,121].

There are also clinical examples of directly combining drugs with probiotics, leading to significant breakthroughs in disease treatment. Recently, a phase 1 clinical trial (NCT03829111) showed that when patients with metastatic renal cell carcinoma (mRCC) received treatment with nivolumab + ipilimumab, combined with the probiotic oral drug CBM588, the median progression-free survival (PFS) was greatly improved, with a median PFS extension from 2.5 to 12.7 months, an increase of nearly 400%[122]. For the first time, it has been demonstrated that oral probiotic drugs can improve the gut microbiota homeostasis of cancer patients and enhance immune therapy response. Immune checkpoint inhibitors, such as CTLA-4 and PD-1, are powerful tumor immunotherapy agents. However, their clinical use can disrupt the immune balance, causing side effects like diarrhea, colitis, and even severe autoimmune

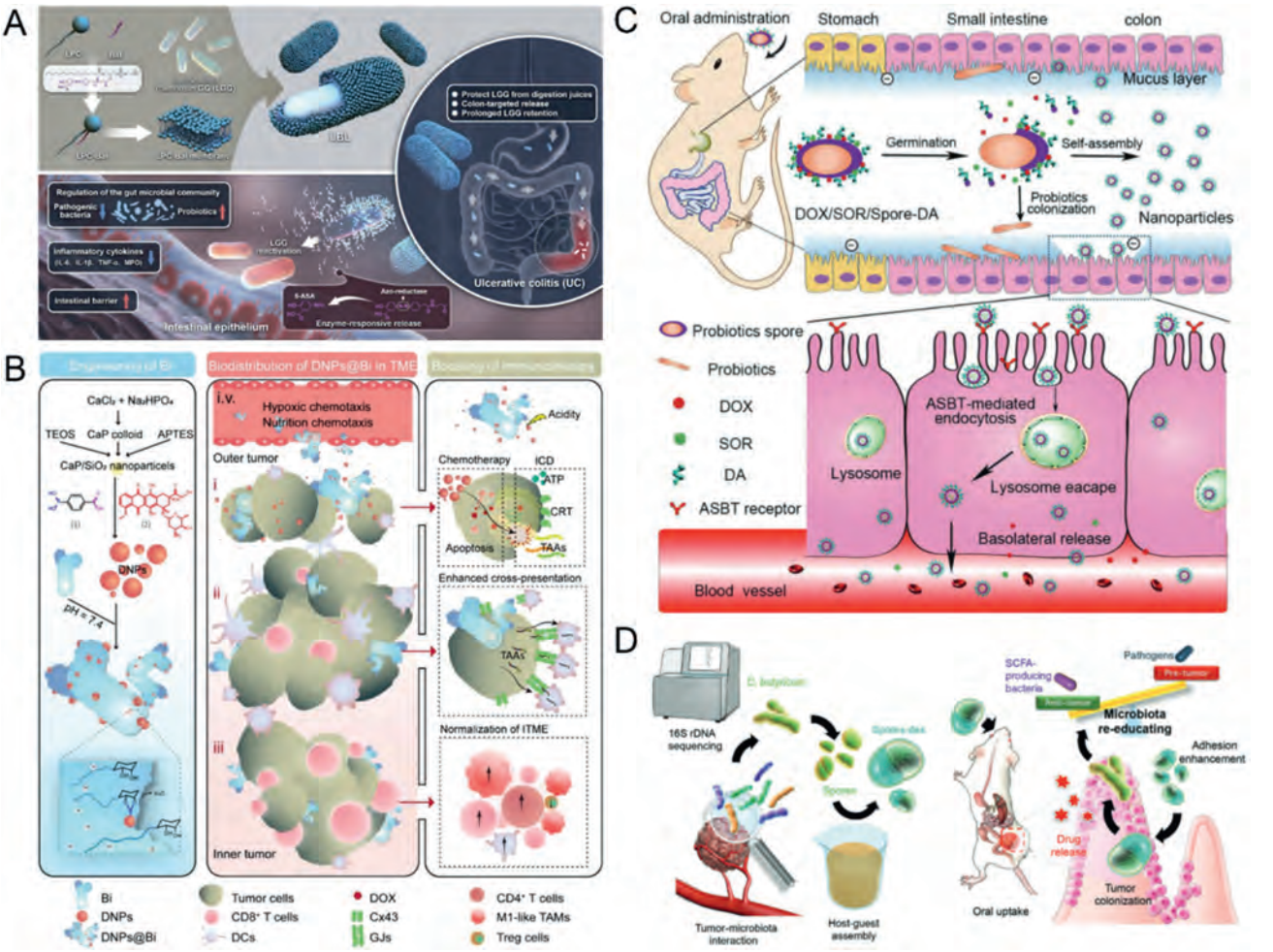

Figure 7 Combination of modified probiotics and pharmacotherapy. (A) Prodrug integrated envelope on probiotics to enhance target therapy for ulcerative colitis. Reprinted with the permission from Ref. 71. Copyright © 2023, Wiley-VCH. (B) Enhanced immunogenic cell death and antigen presentation *via* engineered *Bifidobacterium bifidum* to boost chemo-immunotherapy. Reprinted with the permission from Ref. 114. Copyright © 2023, American Chemical Society. (C) A probiotic spore-based oral autonomous nanoparticles generator for cancer therapy. Reprinted with the permission from Ref. 119. Copyright © 2019, Wiley-VCH. (D) Prebiotics-encapsulated probiotic spores regulate gut microbiota and suppress colon cancer. Reprinted with the permission from Ref. 120. Copyright © 2020, Wiley-VCH.

reactions. Sun et al.[123] revealed that *Bifidobacterium* optimize the gut microbiota composition and enhance the function and metabolism of intestinal regulatory T cells (Tregs) under CTLA-4 blockade, effectively reducing intestinal inflammation caused by anti-CTLA-4 antibodies.

3.2. *Combination of modified probiotics and phototherapy*

Photodynamic therapy (PDT) as an effective cancer treatment is being pursued with enthusiasm[124]. The TME responsive photosensitizers (PSs) are necessary for the tumor-targeted precise PDT. However, many obstacles hinder the widespread application of PS-based therapies, such as the limited tumor penetration and retention, rapid weakening of the photodynamic effect, and the need for multiple irradiations for combination therapies[125,126]. Since probiotics tend to flow into the hypoxic and immunosuppressive TME, probiotic-based tumor-targeted therapeutic systems have been developed in recent years.

As shown in Fig. 8A, Guo and colleagues[127] used surface modification and gene expression to construct a monochromatic radiation-based ternary system that combined photoacoustic and photothermal techniques within BL21 cells. They explored its application in photoacoustic imaging-guided synergistic photothermal therapy in tumors. Indocyanine green and PDA nanoparticles were co-deposited on the surface of the melanin-expressing bacterium BL21 by *in situ* polymerization. The integrated BL21 could produce stable triple photoacoustic and photothermal effects after monochromatic irradiation, owing to the adequate absorption capability of melanin, indocyanine green, and PDA at 808 nm. This opened up a fresh possibility for the creation of novel photosensitizers. Intratumoral injection of the ternary photosensitizer combined with BL21 showed a considerable improvement in tumor regression and extended survival in colon cancer and breast cancer-bearing mice.

Yang et al.[128] coupled *Lactobacillus acidophilus* (LA) with 2D CoCuMo layered-double-hydroxide (LDH) nanosheets (LA&LDH) for tumor-targeted precise NIR-II photodynamic treatment. Following tail vein injection, LA induced low pH (5.4), and the LA metabolites produced glutathione (GSH) in TME may etch 2D CoCuMo-LDH nanosheets. When exposed to 1270 nm laser

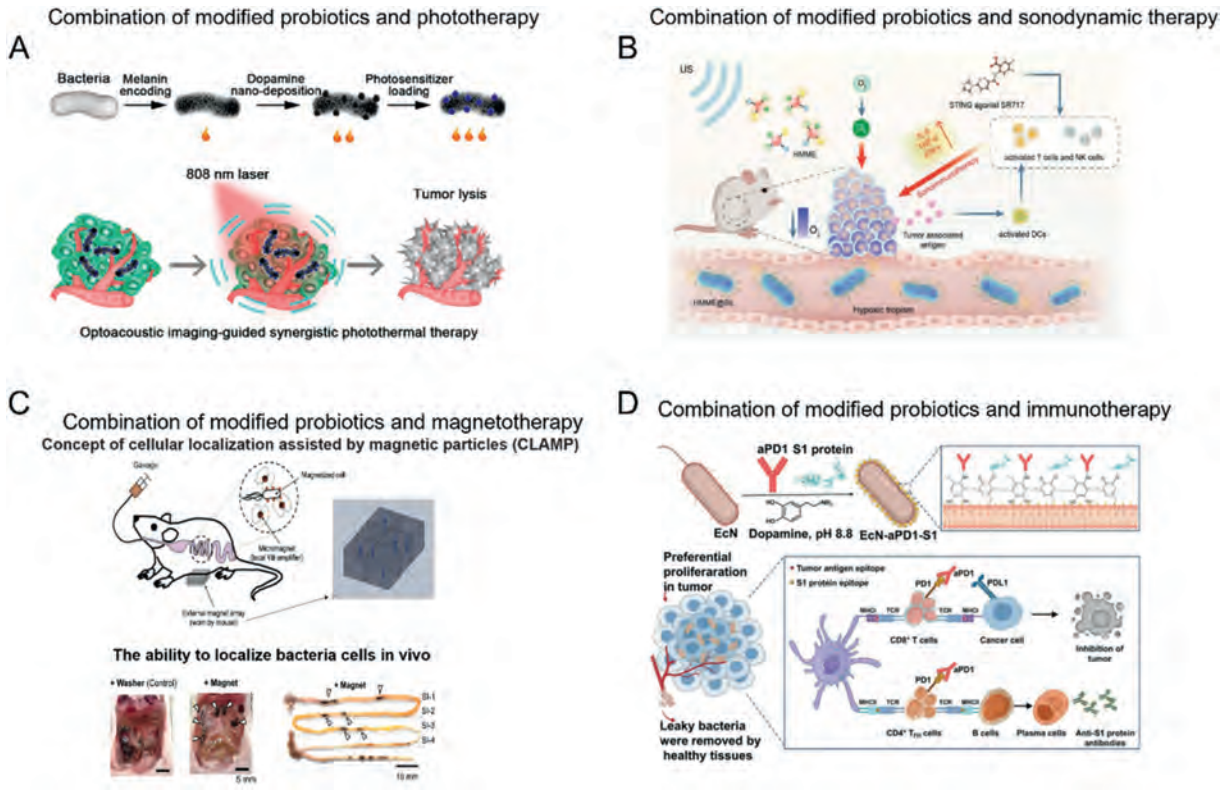

Figure 8 Combination of probiotics with phototherapy, sonodynamic therapy, magnetotherapy and immunotherapy. (A) Optoacoustic imaging-guided synergistic photothermal therapy. Reprinted with the permission from Ref. 127. Copyright © 2023, Wiley-VCH. (B) Probiotic-microbe-based SIT against cancer with STING agonist. Reprinted with the permission from Ref. 134. Copyright © 2022, Wiley-VCH. (C) Spatial control of probiotic in the gastrointestinal tract assisted by magnetic particles. Reprinted with the permission from Ref. 136. Copyright © 2021, Wiley-VCH. (D) Dressing bacteria with a hybrid immunoreactive nanosurface to elicit dual anticancer and antiviral immunity. Reprinted with the permission from Ref. 140. Copyright © 2023, Wiley-VCH.

irradiation, the phase transition of the CoCuMo-LDH nanosheets from crystalline to amorphous significantly increased their ROS generation activity and achieved complete tumor elimination effectively. This work showed probiotics could be employed as a tumor-targeting platform for effective and precise NIR-II PDT.

Shi et al.[129] combined D-alanine (d-Ala) as the metabolite of *C. butyricum* with aggregation-induced emission (AIE) photosensitizer TPApy to create an engineered *C. butyricum* to cure melanoma. Once injected into melanoma, the modified *C. butyricum* increased only in the anaerobic region, enhanced the tumor immune microenvironment, and destroyed the hypoxia region. Following that, the relatively high oxygen level in the peripheral zone triggered the death of *C. butyricum*. Under light irradiation, the PSs on the bacteria exerted a photodynamic impact in the oxygen-rich area, further eliminating melanoma residues.

Exploring novel probiotics to regulate the gut microbiota holds significant therapeutic promise because the discovery of the gut−brain axis has demonstrated the capacity of gut microbial metabolites to impact brain function. In PD, a common neurological illness, dopaminergic neurons in the substantia nigra deteriorate, which results in both motor and non-motor symptoms. PD patients frequently have gastrointestinal problems. Gut microbiota and their metabolites, such as SCFAs and gamma-aminobutyric acid (GABA), communicate with the brain through many ways (endocrine pathway, immune system, nerve pathway), which is discovered with the elucidation of the gut−brain axis. As a result, probiotics that reshape gut microbiota will be crucial in treating PD.

Pan et al.[130] developed three blue-light-responsive probiotics as orally live biotherapeutics. The three engineered *Lactobacillus* strains were designed to produce GABA, granulocyte colony-stimulating factor (GCSF), or GLP1 upon exposure to blue light to modulate anxiety disorders, PD, or neural signaling pathways, respectively. The micro-nano system targeted the small intestine and promoted the abundance of exogenous *L.lactis* in the intestines. This achieved accurate control of mental processes such as anxiety, PD, and vagal nerve stimulation.

In addition to creating probiotics that respond to blue light, Zhang et al.[131] also created an EcN 1917 strain (ROEN) with a red light-responsive controlled drug release profile. ROEN enclosed in pH-sensitive alginate hydrogel microcapsules (AMCs) was given orally to target the intestines. After oral administration, ROEN released Exendin-4 under optogenetic control in the PD mouse model. The released Exendin-4 fused with the anti-neonatal Fc receptor affibody, which endowed ROEN with the function of regulating the gut−brain axis. This red-light optogenetic probiotic offered a novel drug delivery and gut−brain axis regulatory platform.

3.3. Combination of modified probiotics and sonodynamic therapy

There have been several research on using ultrasound (US) in detecting and treating diseases since light has a limited ability to penetrate human tissues, whereas ultrasound has a high penetration rate. Sonodynamic therapy (SDT) is a low-frequency

ultrasound-mediated, non-invasive physical stimulation therapy with high biocompatibility. Hematoporphyrin monomethyl ether (HMME) has been approved for clinical use in treating tumors. Still, its low molecular weight, as well as poor tumor enrichment and retention, severely limit the performance of HMME-based sonodynamic therapy. In addition, due to the immunosuppressive nature of tumor tissues, the immunogenic anti-tumor efficacy of SDT is low[132]. In contrast, probiotics can accumulate and retain in hypoxic tumor cores. It has been shown that *Bifidobacterium* preferentially accumulates in tumors when it enters the bloodstream. This is likely because the hypoxic microenvironment is a tropism, and the immunosuppressive circumstances encourage bacterial colonization and growth[133].

In Fig. 8B, Lu et al.[134] grafted the sonosensitizer HMME onto the specialized anaerobic probiotic *Bifidobacterium longum* (BiL) to create HMME@BiL, which achieved the tumor-targeted HMME delivery with the help of the anaerobic tendency of BiL. This improved the accumulation of sonosensitizers in tumor location, resulting in a practical SDT effect. Simultaneously, the STING agonist SR717 promoted anti-tumor immunity by encouraging the tumor infiltration of cytotoxic T-cells and NK cells and releasing associated cytokines, making sonoimmunotherapy (SIT) possible. In the CT26 colorectal cancer metastasis model, the experimental results showed that combination treatment of HMME@BiL + US + SR717 could successfully inhibit primary tumors and reduce the course of the disease.

However, many modified probiotics can release payloads outside the tumor site after injection, potentially harming healthy tissues. H. Abedi et al.[135] created a focused ultrasound-assisted controlled administration of therapeutic microbes to overcome this constraint. Focused ultrasound is one form of energy that can be applied non-invasively to specific anatomical regions, including solid tumors. The research developed genetically engineered EcN that express temperature-sensitive gene regulatory switches and tumor-suppressive nanobodies. The engineered EcN expressed nanobodies to kill tumor cells when the tumor site was heated by focused ultrasound. By introducing temperature-dependent genes and nano-antibody genes, the scientists created bacterial strains that manufactured tumor-suppressing nanoantibodies when heated to a trigger temperature of 42−43 °C. These strains did not induce anti-tumor nano-antibodies when put into the body since the usual body temperature is 37 °C. Instead, they developed invisibly within the tumor up until a heat source outside of it raised them to the trigger temperature. This technology provided a pivotal tool for the spatiotemporal targeting of potent probiotics therapies in multifarious biological and clinical scenarios.

3.4. Combination of modified probiotics and magnetotherapy

Although engineered probiotics are adaptable, their unpredictability *in vitro* and inaccurate *in vivo* localization are drawbacks. Therefore, techniques that enable the controlled long-term localization of exogenous probiotic agents *in vivo* are required. The magnetic field is an ideal control mechanism since it can freely penetrate biological tissues. However, magnetic fields, like the GI tract, are challenging to apply with enough strength to directly manipulate magnetically tagged cells in deep tissue. As shown in Fig. 8C, Buss et al.[136] attempted the cellular localization assisted by magnetic particles (CLAMP) method. When given orally and combined with an applied magnetic field, a composite biomagnetic material made of probiotics (EcN BL21 and EcN 1917) and microscale magnetic particles (carboxyl-functionalized superparamagnetic iron oxide particles) allowed probiotics to be captured and retained in the gastrointestinal tract of mice. This technology enhanced the accumulation and colonization of probiotics at targeting locations. This cellular localization provided external physical control to an important developing class of microbial therapies.

3.5. Combination of modified probiotics and immunotherapy

Immunotherapy, as a powerful clinical strategy for treating cancer, has some drawbacks, such as the relatively low response rate and severe side effects, such as autoimmunity and nonspecific inflammation[137]. Either of these will severely limit immunotherapies' efficiency[138]. Many delivery materials appeared to enhance the immune response and weaken the side effects. Various nanoparticles and even T cells are used to convey therapeutics to target the tumor area, reduce systemic immune toxicity, and decrease the accumulation of non-target sites[139]. Probiotics, especially those anaerobic probiotic bacteria, have a particular characteristic: the natural tendency to hypoxic tumors, making them excellent candidates for transmitting immunotherapies. As a result, the combination of probiotics and immunotherapy emerged.

Li et al.[139] reported an approach of decorating probiotic EcN with triple immune nanoactivators to develop tumor resident living immunotherapeutics. The tumor-specific antigen OVA and immune checkpoint inhibitor α-programmed cell death protein (PD) 1 were conjugated to PDA nanoparticles. Then, these conjugators were attached to the bacterial surface *via in situ* precipitation polymerization of dopamine. Such decorated probiotics show spatiotemporal tumor retention and proliferation-dependent drug release. In addition to acting as a connector, PDA's photothermal effect could switch tumor-associated macrophages M2 to a pro-inflammatory M1 phenotype. The OVA antigens stimulated the DCs and initiated immune responses specific to the tumor. At the same time, the anchored immune checkpoint inhibitor α-PD-1blocked immune checkpoints and activated CTLs. This research created a promising platform to combine probiotics with immunotherapy.

In Fig. 8D, Liu et al.[140] coated EcN with a hybrid immunoreactive nanosurface using the same method: one-step *in situ* polymerization with the polymerization of dopamine. Specifically, the complex of a checkpoint-blocking antibody and a virus-specific antigen covalently conjugated to PDA nanoparticles were deposited on the probiotic EcN, termed EcN-αPD1-S1. Given the natural tendency to hypoxic tumors of EcN, the coated probiotic enabled sustained release and improved exposure of carried αPD1 and S1 protein for long-acting stimulation of immune cells.

3.6. Other applications for bioimaging and diagnosis

Probiotics can colonize *in vivo* biointerfaces, particularly the oral mucosa, reproductive tract, and gastrointestinal tract, and target specific lesion sites such as tumors. Under their inherent features, such as mobility, editability, and targeting, probiotics integrated with imageable agents are frequently used as living probes for bioimaging and disease diagnosis[17,141]. As the primary parts of mammalian microbiomes, both Gram-positive and Gram-negative bacteria can colonize various biological interfaces *in vivo* and impact human health and even the course of diseases[142].

As shown in Fig. 9A, Jiang et al.[143] developed a genetically engineered probiotic strain EcN 1917 by coexpressing the genes firefly luciferase (Fluc) and luciferin-regenerating enzyme (LRE) to produce robust, consecutive, and red-shifted bioluminescence

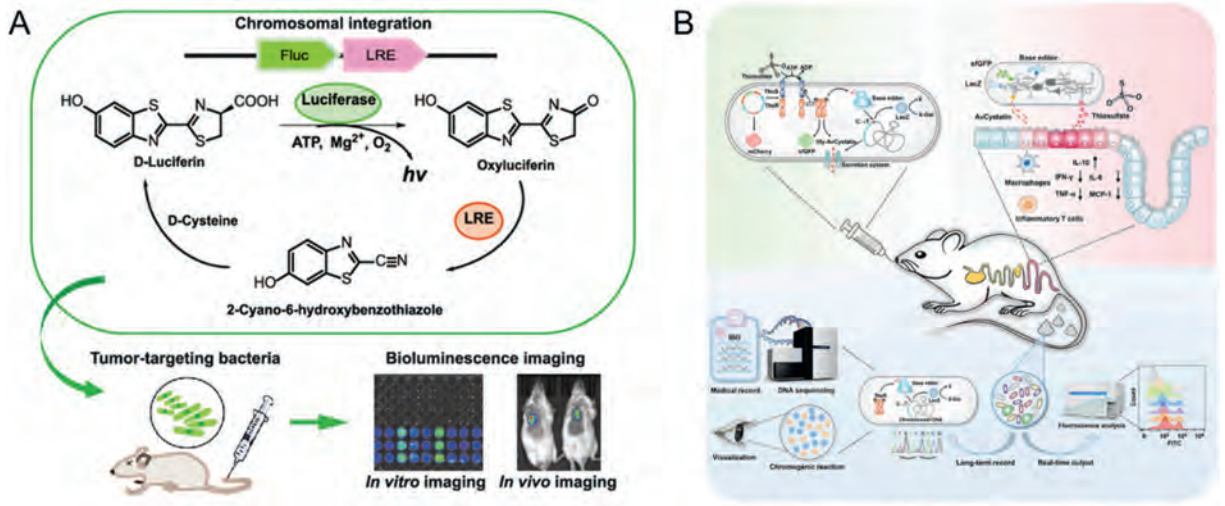

Figure 9 Probiotic-based bioimaging and diagnosis. (A) EcN was engineered to contain the core module Fluc-LRE. Reprinted with the permission from Ref. 143. Copyright © 2021, American Chemical Society. (B) Schematic diagram of the components and sensory pathways of i-ROBOT. Reprinted with the permission from Ref. 144. Copyright © 2023, Elsevier.

for bacteria-tracking both *in vitro* and *in vivo*. This study provided an optical *in vivo* tumor-targeting system for investigating bacteria-associated cancer therapy.

The intelligent whole-cell biosensors constructed *via* synthetic biology have been extensively studied recently. In Fig. 9B, Zou et al.[144] constructed an intelligent probiotic (i-ROBOT) to diagnose, record, and ameliorate IBD. The main component of i-ROBOT was probiotic EcN 1917, which could respond to different levels of the inflammatory marker thiosulfate by stimulating a base-editing system to produce a heritable genomic DNA sequence and generate a colorimetric signal simultaneously. The adjustable release of the immunomodulator *Acanthocheilonema viteae* cystatin (AvCystatin) was also driven by fluctuations in thiosulfate. After oral administration of i-ROBOT in colitis mice, molecular recording signals were generated in processed fecal and colon samples, and the disease was ameliorated effectively. i-ROBOT offered a promising platform for gastrointestinal disorders and other metabolic diseases.

Although numerous studies have highlighted the crucial role of modified probiotics in combination with other treatments for various diseases and in bioimaging and diagnosis, their biosafety remains a significant concern, especially that of modified probiotics. Modification may alter the natural behavior of probiotics, enabling them to more easily adhere to epithelial cells or resist normal clearance processes, thereby increasing the risk of unintended colonization or pathogenicity. Some modified probiotic strains may transfer genes to gut microbes, potentially contributing to antibiotic resistance or other health risks[145,146]. For populations with HIV, cancer, or individuals undergoing organ transplants or receiving immunosuppressive drugs, the weakened immune response can reduce the body's ability to contain and eliminate live microbial agents, increasing the risk of infections. Immunocompromised individuals may be more susceptible to infections such as bacteremia (bacterial infections in the bloodstream) or sepsis if the probiotic strains translocate from the gut into the bloodstream. Cases of sepsis associated with *Lactobacillus* and other common probiotic strains have been reported in severely immunocompromised patients[147].

In light of these risks, it is essential that studies on probiotics—especially modified ones—include robust safety assessments tailored to vulnerable populations. Regulatory guidelines could also mandate testing protocols that better predict the safety of probiotics in immunocompromised individuals, helping to balance the benefits and risks across different groups.

4. Modified probiotics in clinical trials

Although numerous fundamental studies that employ modified probiotics to solve the above problems have been reported, moving the physicochemical modified probiotics or biomedical modified

Table 3 Examples of genetic engineering probiotics in clinical trials.

Engineered probiotic	Disease	Company/university	Stage	ClinicalTrials.gov identifier
B. longum to deliver the plasmid for IL-12 expression	Solid tumors	IQVIA	Phase 1	NCT04025307
EcN 1917 to target STING-activation	Solid tumors	Synlogic	Phase 1	NCT04167137
EcN 1917 to metabolize ammonia	Cirrhosis and hepatic insufficiency	Synlogic	Phase 1/2	NCT03447730
L. lactis to secrete human Trefoil factor 1	Oral mucositis in patients with head and neck cancer	Actobio therapeutics/ Oragenics	Phase 2	NCT03234465
L. lactis to deliver human proinsulin and hIL-10	Type 1 diabetes	Actobio therapeutics	Phase 2a	NCT03751007
EcN 1917 to metabolize phenylalanine	Phenylketonuria	Synlogic	Phase 3	NCT03516487

probiotics from the laboratory to the clinic remains challenging. This may be attributed to the incomplete understanding of specific key properties of probiotics and the lack of comprehensive clinical trials. Since there are barely any clinical studies about applying physicochemical-modified probiotics, we listed the clinical studies on genetic engineering probiotics here in Table 3.

5. Conclusions and outlooks

Due to their innate qualities, probiotics present enormous potential for biomedical applications, including disease treatment, bioimaging, and diagnosis. However, their high sensitivity to environmental changes hinders the application. Various modification technologies are being developed to convey and protect probiotics from serious situations while generating significant therapeutic effects and lowering side effects. Moreover, combining probiotics and other therapeutics further amplifies the therapeutic effect, making it come to the fore.

Several clinical studies confirmed that probiotics are irreplaceable in treating specific diseases[148]. Due to obstacles in modified probiotics' stability, efficacy, and safety, translating them into clinical applications remains challenging. The variability and complexity of the gastrointestinal environment affect the stability, colonization and therapeutic efficacy of modified probiotics. In addition, ensuring that probiotics deliver therapeutic agents precisely to targeted sites, such as inflamed tissues or tumors, while maintaining bioavailability is a critical yet challenging aspect of clinical application. Then, the systemic administration of bacteria vector at the wrong dose might lead to unwanted adverse cardiovascular complications. Addressing these challenges is crucial for the effective clinical translation of modified probiotics, and we should focus research on improving stability, targeting precision, and establishing clear regulatory pathways.

This article provides an overview of probiotic modification strategies and introduces an innovative perspective by proposing the combinatorial therapeutics of modified probiotics with other therapeutic approaches. With constantly emerging fundamental studies in this new interdisciplinary research field, modified probiotics will open new avenues for fundamental studies and medical applications.

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Author contributions

Lei Wang, Qianhua Feng and Luo Zhao chose the topic and designed the outline for this review. Luo Zhao and Mengya Niu wrote the manuscript. Zilin Ma and Fengyun He drew the figures and designed the tables. Xinxin Liu, Xunwei Gong, Zhanfei Chai and Ziqing Wang revised the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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