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Review Article

Advances in microbial decorations and its applications in drug delivery

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ABSTRACT

Microorganisms are mostly distributed on the surface of our skin and intestines and have crucial roles in physiologic and metabolic processes, such as digestion and immunity, which are closely related to diseases. Recently, microorganisms have received great attention and have been applied in various aspects of biomedicine, especially in the field of drug delivery. However, the application of bacteria has been largely limited due to the intrinsic nature of bacteria, including rapid proliferation, toxicity, and immunogenicity. Therefore, microbial decoration is an attention-grabbing approach to drug delivery by altering the properties and functions of microbial surfaces. Microbial decoration methods are diverse and include biotin-affinity and gene decoration technologies. These approaches can improve the specific delivery of drugs, enhance the stability and controlled release of drug delivery vehicles, and are useful in cancer therapy, gene therapy, and vaccine delivery. Microbial decoration has broad application prospects by helping develop smarter and more precise drug delivery systems and providing more effective and safer therapeutic options for patients. In this review we summarize the research progress in different microbial surface modification methods and the applications in drug delivery, as well as the outlook for future opportunities in this field.

Keywords: microbe, decoration, drug delivery, biotin-affinity technology, targeted therapy

1. INTRODUCTION

Drug delivery is an important topic in modern medicine. Drug delivery involves the effective delivery of drugs to specific parts of the body for therapeutic purposes. However, traditional methods of drug delivery have limitations, such as low drug bioavailability, high side effects, and inadequate local concentrations. Therefore, scientists are striving to find innovative drug delivery strategies to improve drug efficacy and reduce patient discomfort [1-3]. Modern drug delivery research is focused on improving the specificity and efficiency of drug delivery, as well as enhancing drug stability and controlled release to achieve more precise therapeutic effects. Nanotechnology, carrier technology, and biodecoration are among the approaches used by researchers to design and construct drug carriers with optimal delivery properties [4]. These advances facilitate accurate drug delivery and release. Keeping abreast of the latest trends and research advances in drug delivery is crucial to promoting the development of innovative drug delivery systems and providing more precise and efficient methods for clinical treatment [5, 6]. It is expected that this review will provide scientists with new inspiration and insight, leading to advances in the field of drug delivery and ultimately improving therapeutic effects and patient quality of life.

Microbial decoration has attracted extensive attention and research as an innovative drug delivery strategy [7, 8]. Through genetic engineering techniques and biochemical approaches, it is possible to alter the surface properties and functions of microorganisms to facilitate the delivery of drugs and overcome a number of traditional drug delivery method limitations [9]. There are various methods by which to decorate microbes, including biotin-affinity and gene decoration technologies

[10, 11]. Biotin-affinity technology utilizes specific interactions between biotin and affinities to achieve attachment and delivery of drug molecules [12]. Through the steps of constructing biotinylated vectors, expressing and displaying biotin-binding proteins, decoration of the microbe surface can be achieved, enhancing the specificity and stability of drug delivery [13-15]. Genetic decoration technology, in contrast, enhances the efficacy of drug delivery vectors by altering the genetic material of microorganisms so that the microbes possess specific functions and properties. Microbial decoration has broad application prospects in the field of drug delivery. First, microbial decoration improves the specific delivery of drugs, ensuring that drugs are accurately delivered to target tissues or cells, thus improving therapeutic effects. Second, microbial decoration enhances the stability and controlled release of drug delivery carriers, which prolongs the duration of drug action and reduces the toxic side effects of drugs [16, 17]. In addition, due to the flexibility of microbial decoration technology, microbial decoration can be applied to different types of drugs and play a role in several fields, including cancer therapy, gene therapy, and vaccine delivery. Through in-depth research on microbial decoration, we expect to develop smarter and more precise drug delivery systems to provide more effective and safer treatment options for patients [18, 19]. Therefore, microbial decoration research will further promote innovation and progress in medicine and make significant contributions to human health.

Microbe chemical decoration methods are essential strategies for altering microbe surface properties and enhancing the targeting and selectivity of drug delivery systems [20, 21]. The various chemical decoration methods have different advantages and adaptability. Choosing the appropriate decoration method based on specific requirements can achieve precise drug delivery and improve therapeutic effects. However, it is important to consider the reaction conditions and stability of the microbe surface in practical applications to facilitate the widespread use of microbe chemical decoration methods in the field of drug delivery. Microbial decoration in drug delivery offers the advantage of targeted delivery utilizing microbial surface receptors or specific cell recognition mechanisms [22, 23]. This precision enhances drug targeting and selectivity, while also providing protection to drug molecules, increasing *in vivo* stability, and mitigating the risk of degradation due to the resilience and resistance of microorganisms. Additionally, microbial decoration facilitates controlled drug release through microbe cell metabolism and secretion mechanisms, thereby improving release efficiency and duration [24, 25].

2. MICROBIAL DECORATION METHODS

Microbial decoration entails the use of special structures and functions on microbe surfaces, such as bacteria, to introduce ligands, antibodies, or other biomolecules for the purpose of enhancing the targeting and selectivity of drug delivery systems [26]. The selection and application of microbial decoration methods have integral roles in achieving efficient drug delivery.

2.1 Surface decoration methods

2.1.1 Chemical decoration. Chemical decoration involves the covalent attachment [27] of specific compounds, such as ligands [28], antibodies, or chemical primers, to the microbe surface through chemical reactions. Common chemical decoration methods include acylation, activated esterification, click chemistry, and hatching. These methods achieve highly specific drug delivery and targeting but are still restricted by microbe surface structures and chemical reaction conditions.

(1) Activated esterification

Activated esterification is a prevalent microbe chemical decoration method in which esterification reactions [29] occur between activated esterification reagents and amino acids or hydroxyl groups, which are the functional groups on the microbe surface. This method enables the covalent attachment and fixation of drug molecules, thereby altering the surface properties of microbes. By selecting appropriate activated esterification reagents and reaction conditions, highly specific and stable decoration effects can be achieved. For example, Petri and colleagues [30, 31] showed that optimized α-phenyl-α-diazoamides esterifies carboxyl groups in protein targets, such as T cells, under mild aqueous conditions.

(2) Acylation

Acylation is a method in which the anhydride from a compound reacts with amino acids or other functional groups on the microbe surface [32]. Common acylation reagents include anhydrides and carbonates. Acylation reactions can occur under neutral conditions and selectively introduce specific compounds or functional groups. This method can alter microbe surface functions, thus enhancing the specificity and activity of drug delivery. In the study undertaken by Recke and colleagues [33], lipases from the Antarctic yeast and Mucor-Miehei were utilized to connect mannose-based Rhizobium lipids (MEL-A and MEL-B) with unusual hydroxyl fatty acids secreted by other microbes or other microbe glycolipids. An optically active (R)-3-hydroxydecanoic acid was first obtained, and the original glycolipids were enzymatically modified with lipases [33]. Recke and colleagues [33] successfully transferred unusual hydroxy fatty acids to natural glycolipids using biocatalytic methods. Successful acylation was achieved by linking the C-1 position of mannose-based Rhizobium lipids (MEL-A) with 3-OH.

Yang et al. [34] proposed that histone acetyltransferases (HATs) target lysine residues in the histone tail chains during histone acetylation decoration. HATs catalyze the acetylation between the acetyl group in the compound and the lysine residue through acylation

reactions, which leads to a change in the charge state of lysine. Acetylated lysine loses its positive charge, thereby weakening the ability of histones to bind to the negatively charged DNA backbone. WareJoncas et al. [35] suggested that fusion of the DNA binding system with HATs or histone deacetylases (HDACs) enables programmable acetylation or deacetylation of specific lysine residues on histones associated with target DNA. Therefore, acetylation indirectly affects DNA expression by modulating protein-protein interactions and increasing accessibility to the target area. Overall, histone acetylation and deacetylation are crucial modification methods for gene expression regulation. Acetylation indirectly influences DNA expression by altering the charge state of lysine. Consequently, in-depth studies on the specificity of acetylation targeting epigenetic technologies are important.

(3) Click chemistry

Click chemistry is an efficient chemical decoration method. Click chemistry realizes the covalent attachment of compounds through the use of click reagents that have high selectivity with the functional groups on the microbe surface [36]. Common click chemistry reactions include the copper-catalyzed cyclo-addition reaction of arylacetylene with azides (CuAAC) and the tetrazole cyclo-addition reaction (Tz-click). Click chemistry holds the advantage of rapidity, high specificity, and high yields, thus finding broad application in microbial decoration. Hatzenpichler and colleagues [37] introduced a novel click chemistry technique (BONCAT), as shown in **Figure 1**. This technique uses non-natural amino acid labeling and click chemistry methods to visualize newly synthesized proteins in environmental samples and understand protein conditions within individual microbes. The study adopted the non-standard amino acid, L-azidohomoalanine (AHA), to replace natural methionine, followed by a reaction with a click chemistry reagent containing the alkyne nitrogenous heterocyclic ring to fluorescently label cell proteins containing AHA. The study also combined BONCAT with ribosomal RNA (rRNA)-targeted fluorescence *in situ* hybridization to directly associate taxonomic identity with translational activity. The potential of BONCAT in studying microbes and their responses to environmental stimuli was demonstrated by performing BONCAT experiments on methane-oxidizing enrichment cultures incubated under different conditions. BONCAT has advantages compared to other single-cell metabolic activity indicators, such as easy operability, cost effectiveness, and facilitating the direct observation of newly synthesized proteins.

2.2 Microbe molecular crosslinking

Microbe molecular crosslinking is a method that uses specific biomolecules to bind to receptors on the surface of microbes, such as bacteria, forming stable crosslinks and interactions. Through microbe molecular cross-linking, selectivity, and targeting of drug delivery systems can be enhanced. This review will provide a comprehensive overview of various methods of microbe molecular crosslinking and explore their applications in the field of drug delivery.

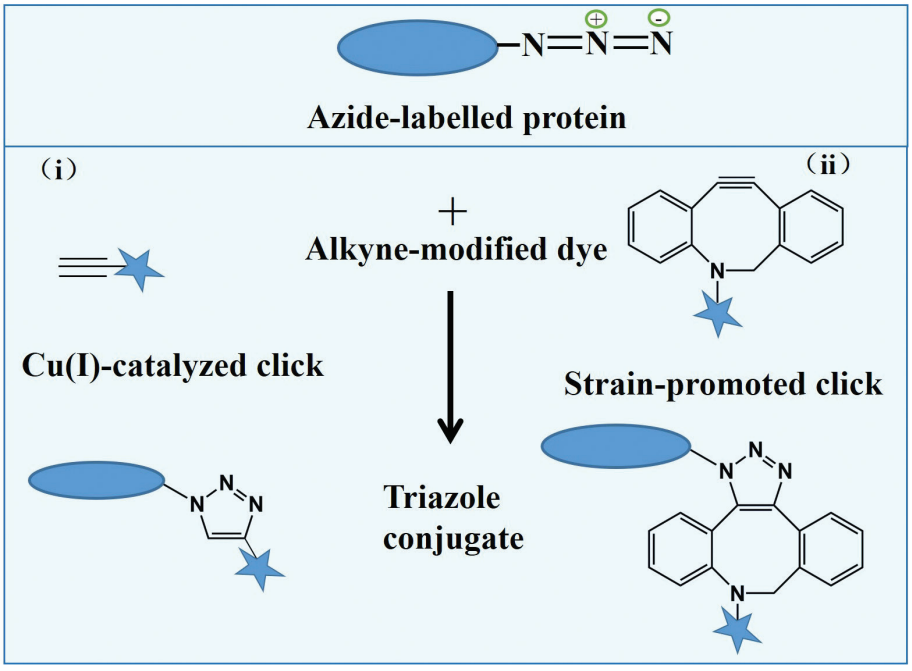

Figure 1 | The click chemistry-mediated of newly-produced AHA-containing proteins [37].

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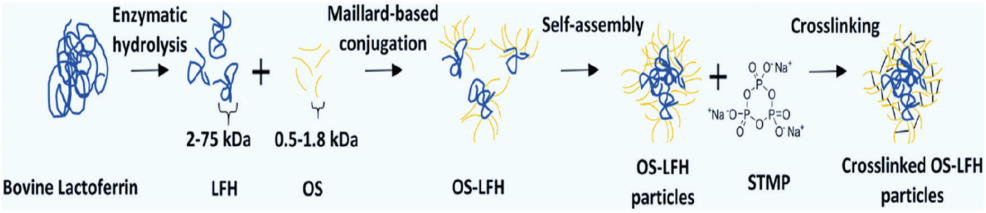

Figure 2 | Schematic formation process and structure of the OS-LFH shell crosslinked particles [38].

2.2.1 Affinity ligand crosslinking. Affinity ligands, as a class of biomolecules, stably bind to receptors on the microbe surface and form specific crosslinking. Common affinity ligands include antibodies, receptor ligands, and oligonucleotides. By utilizing affinity ligand crosslinking, highly selective binding of drug delivery systems to specific cells, tissues, or molecular targets can be achieved, thereby enhancing drug targeting. Based on a study involving microbe affinity ligand crosslinking, Peled et al. [38] reported a novel protein delivery system using oligosaccharide and lactoferrin hydrolysate conjugates as molecular recognition ligands. Through the preparation of self-assembling micellar particles with three different compositions of prebiotics (oligosaccharide-lactoferrin hydrolysate [OS-LFH], oligofructose [FOS-LFH], and xylo-oligosaccharide [XOS-LFH]), selective targeting delivery to probiotics can be achieved, while accelerating the proliferation of beneficial microorganisms, as shown in **Figure 2**.

These OS-LFH microparticles are promising protein delivery systems. OS-LFH microparticles achieve highly tolerable and selective targeting delivery to intestinal probiotics, thereby enhancing the competitiveness of probiotics and harmful bacteria and promoting improved health. Microbe affinity ligand crosslinking has a crucial role in preparing this protein delivery system. Low polysaccharide-LFH conjugates are prepared and guided to specifically bind to the sugar binding proteins of intestinal probiotics via the molecular recognition ligand, thereby achieving targeted protein delivery. Furthermore, by treating the OS-LFH with the cross-linking agent, STMP, the resulting microparticles exhibit strong structural stability and digestive tolerance. By combining these two key steps, a protein delivery system with good characteristics was successfully prepared. This method is expected to be used in the development of a new generation of protein-containing prebiotic compounds, achieving targeted proliferation of intestinal probiotics.

Continued research and development of microbe affinity ligand crosslinking methods will help drive innovation and improve protein delivery systems, providing new strategies and directions for drug delivery and treatment in the field of biomedicine.

2.2.2 Antibody crosslinking. Antibody crosslinking refers to the use of an antibody with two antigen-binding sites. By binding this antibody to receptors on the microbe surface, specific functions of the microbe surface and the stable binding of drug molecules can be achieved. This method of microbe molecular crosslinking is often used in constructing drug delivery carriers, endowing drug delivery carriers with high selectivity and a high capacity for drug molecules.

In studies on dual-antibody crosslinking, Gaspar et al. [39] described FS120 mAb2, a dual agonist bispecific antibody targeting CD137 and OX40 that activates CD4+ and CD8+ T cells, whereas OX40 or CD137 monospecific antibodies only activate CD4+ or CD8+ T cells, respectively. FcγR-disabling mutations have been introduced to enable antibody crosslinking from co-engagement of two different receptors when co-expressed and to potentially avoid depletion of OX40- or CD137-expressing cells. A mouse-specific surrogate version of FS120 has been shown to have antitumor activity in the absence of FcγR interaction or after Treg depletion.

The design of bispecific antibody crosslinkers offers a novel approach and methodology for drug delivery systems. By exploiting the interaction between bispecific antibodies and receptors on the microbe surface, higher selectivity and remarkable payload capacity for drug molecules can be achieved, thus providing robust support for precision medicine. Future research endeavors aiming to further explore and optimize the mechanisms and applications of bispecific antibody crosslinkers could pave the way for more innovations and breakthroughs in the development of drug delivery systems and antibody therapy within the biomedicine field.

2.2.3 DNA complementary pairing. The principle of DNA sequence complementary pairing can be utilized to crosslink DNA sequences with specific functions to complementary DNA sequences on microbe surfaces. This method allows for the introduction of specific functional groups or targeting sequences on microbe surfaces, thereby ensuring the specificity and targeting of drug delivery. Additionally, DNA complementary pairing can be utilized to construct robust multi-component microbe systems, enabling complex drug delivery strategies [40].

Molecular crosslinking of microbes represents an effective mode of enhancing the targetability and selectivity of drug delivery systems [41]. Stable binding can be achieved between microbe surfaces and specific molecules or cells through various crosslinking methods,

such as affinity ligand crosslinking, bispecific antibody crosslinking, and DNA complementary pairing, resulting in precision control of drug delivery [42, 43]. While microbe molecular crosslinking methods present ample potential for application in the realm of drug delivery, further research is warranted to optimize the crosslinking methods and improve crosslinking efficiency.

2.3 Biotin-avidin technology

Biotin-avidin technology involves the fusion of biotin with the avidin on the surface of microbes, realizing the attachment and delivery of drugs through the interaction between biotin and avidin. This method has high specificity and controllability, and is commonly used in the building and research of drug delivery systems [44]. Biotin-avidin technology is a common method for microbial decoration, utilizing the strong specific interaction between biotin and avidin, linking biotin to the surface of microbes, such as bacteria, to achieve drug molecule attachment and delivery [45]. The following section will discuss in detail the principles and methods of biotin-avidin technology, and extensively discuss its advantages, limitations, and application cases in the drug delivery field.

Biotin-avidin technology relies on the non-covalent binding between biotin and avidin [46]. Biotin is a small molecule that is widely found in nature and has high stability and specificity [47]. By choosing the appropriate biotin binding protein, such as avidin, to bind with biotin, decoration of the microbe surface can be achieved. Pre-targeted radioimmunotherapy (PRIT) utilizes the extremely high affinity of the avidin-biotin system. The avidin-biotin system has been widely used in many life science fields. Avidin is a glycoprotein with a molecular weight of 66kDa. Streptavidin has a molecular weight of 53kDa and originates from non-glycosylated bacterial protein of the *Streptomyces* genus; both avidin and streptavidin have been used for PRIT [48]. Avidin and biotin have extremely high affinity and specificity, with one avidin protein able to bind to four biotin molecules. The dissociation constant of avidin-biotin complexes is approximately 10^{-15} M, which is 10^5–10^6 times the general antigen-antibody reaction [49]. If the biotin binding site of avidin binds ≥ 2 biotin molecules, steric hindrance can result. Biotin can theoretically bind to antibodies or act as a carrier for transporting radioactive isotopes. Both forms of biotin action have been used in PRIT evaluations.

Jin et al. [50] studied a stimuli-responsive drug delivery system (DDS) that can adjust size and ligand (biotin) according to the tumor microenvironment. As shown in **Figure 3**, in conjunction with the two-step

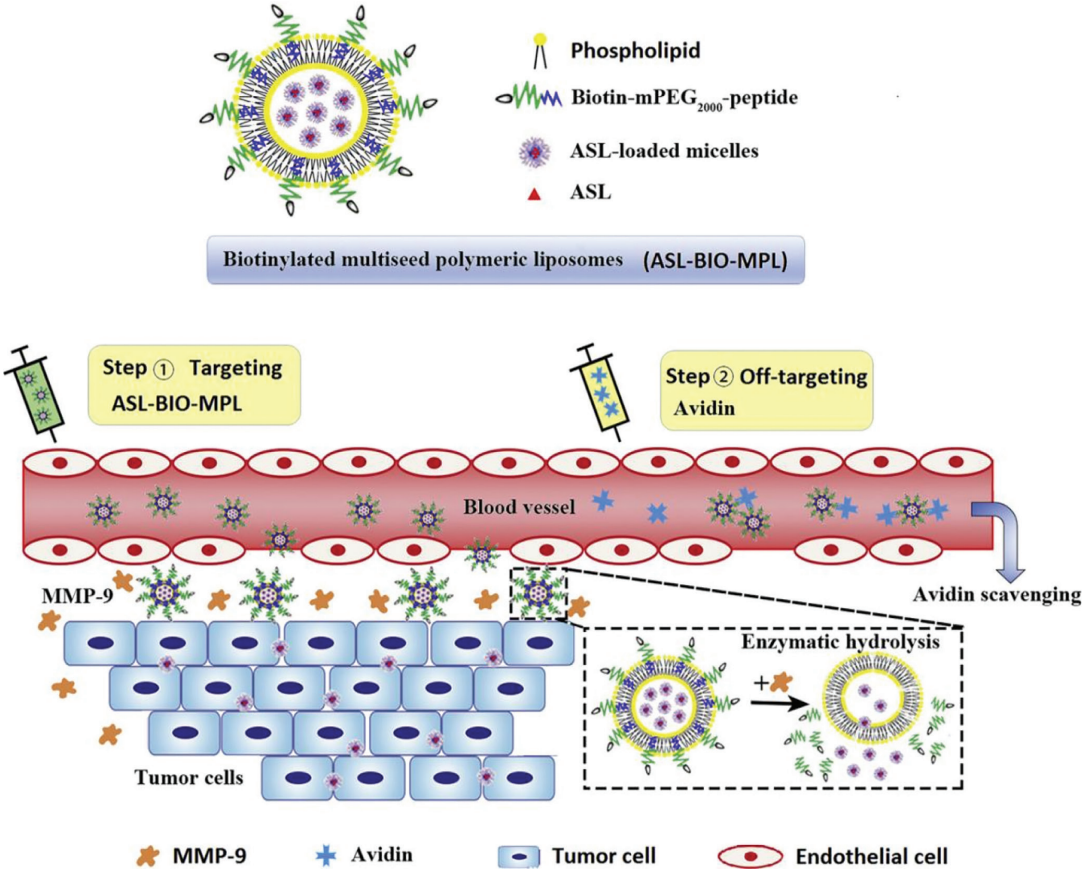

Figure 3 | Schematic design of biotinylated multiseed [50].

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strategy of the biotin-avidin system, a DDS seeks to target tumors, penetrate depth, and purify unresponsive nanocarriers in normal tissue. This DDS is composed of multiple seed-containing aggregate liposomes (ASL-BIO-MPLs), with Nile Red encapsulated in the aqueous phase of the seed micelles. The shell layer of these aggregate liposomes is functionalized by an MMP-9 cleavable polymer-peptide modified with tumor-targeting ligand biotin. After incubation with MMP-9, ASL-BIO-MPLs decompose into a mixture of irregular-shaped liposomes and dispersed small micelles. Fluorescently-labeled BIO-MPLs may take advantage of the central infiltration of the released small micelles into 4T1 breast tumor spheroids. Moreover, compared with other formulations, ASL-BIO-MPLs demonstrated the strongest drug penetration power and the best tumor growth inhibition effect. Affinity consecutively administered in a reasonable dosing scheme, ASL-BIO-MPLs, by reducing the number of apoptotic cells induced in normal tissues without affecting its targeting effect, suggest that subsequent avidin can clear DDS from non-target sites. In summary, this dual-step biotin-avidin strategy based on a size/ligand-adaptable MPL system may provide potential pathways for the penetration and protection of normal tissue by nanocarriers in deep tumor targeting.

2.4 Genetic engineering methods

2.4.1 Gene decoration. Gene decoration, achieved through genetic engineering technology, introduces specific gene sequences into microbes, enabling microbes to express proteins with specific functions or bioactive substances [51]. For example, genes for drug delivery proteins, targeting ligands, or secretion-related enzymes can be inserted into bacteria to facilitate the construction and functional enhancement of drug delivery carriers. Gene decoration is a commonly used microbial decoration method that changes surfaces and functions by introducing, deleting, or altering the genetic material of microbes, such as bacteria [52, 53]. Gene decoration methods are widely used in microbiology and have crucial roles, especially in drug delivery and bioproduction. The following section will detail various gene decoration methods, with a focus on the advantages, limitations, and applications across different fields.

Gene decoration is a key method for microbial decoration where the microbe genome can be altered to manipulate surface properties and functionalities [54]. Gene editing techniques, plasmid transfection techniques and copy number regulation are commonly used gene decoration methods, known for their efficiency, precision, and controllability [55, 56]. While gene decoration technologies have broad application prospects in drug delivery and bio-production, challenges such as transformation efficiency and decoration stability need to be addressed to further advance practical applications.

(1) Gene editing techniques

The CRISPR-Cas system is a widely used method for gene editing and enabling targeted genome cutting, replacement, or insertion by introducing CRISPR RNA and Cas proteins [57, 58]. This method is efficient, precise, and straightforward and has been widely used in microbe gene decorations. Xu et al. [59] have made use of the CRISPR-Cas12f system. By engineering the proteins and RNA of the Cas12f (Cas14) system, Xu et al. [59] created a compact CRISPR-Cas system (CasMINI) for regulating and editing mammalian genomes. Through further protein and RNA engineering, Xu et al. [59] successfully activated genes and demonstrated the substantial efficacy of the CasMINI system in gene and base editing. Experimental results have shown that the CasMINI system increases gene activity 1000s-fold. In addition, the CasMINI system also facilitates efficient and specific gene and base editing. Experimental results indicate that CasMINI has a high efficiency and specificity, and through optimized sgRNA design and multiple protein engineering screening rounds, CasMINI effectively activates gene expression and performs efficient gene and base editing. CasMINI presents a comparable degree of efficient delivery and function as the commonly used Cas9 and Cas12a systems, suggesting considerable potential for gene engineering applications.

(2) Plasmid transfection techniques

Stable expression of exogenous genes can be achieved through the integration of exogenous genes into a plasmid vector followed by the introduction of this plasmid into microorganisms [60]. Common methods include electroporation, heat shock transformation, and chemical transformation. The plasmid transfection technique is widely used to introduce and express foreign functional genes on the surface of microorganisms, thereby changing the function and properties of microorganisms.

Feng et al. [61] synthesized a hyperbranched polymer (HP) with high plasmid DNA (pDNA) binding affinity and negligible cytotoxicity. An HP can self-assemble into nano-sized polymeric clusters with a "double-shell" structure, which efficiently transfers pDNA into NP cells. These polymers were encapsulated in biodegradable nanospheres (NS) to realize two-stage delivery: 1) temporary controlled release of pDNA-loaded polymer clusters; and 2) efficient delivery of pDNA into cells by released clusters. These biodegradable NS were co-injected with nanofiber sponge microspheres (NF-SMSs) to target cell transfection coding for orphan nuclear receptor 4A1 (NR4A1). NF-SMSs have been recently reported as a therapeutic for delaying pathogenic fibrosis. An HP effectively transfects human NP cells *in vitro* with low cytotoxicity. The two-stage delivery system maintained a polymer presence in rat tails for an extended period (> 30 days). In the rat tail degeneration model, we found that the NR4A1 pDNA carried

by HP polymers therapeutically reduced the pathogenic fibrosis of NP tissues. In conclusion, the combination of two-stage NR4A1 pDNA delivering NS and NF-SMSs could suppress fibrosis and support intervertebral disc (IVD) regeneration.

2.4.2 Vector selection and recombination. Vector selection and recombination processes involve choosing specific microbe strains or implementing gene recombination techniques to introduce vectors, such as extracellular vesicles possessing specific functionalities and biological activities into target microorganisms [62]. This approach leverages the unique structures and functionalities of vectors to achieve drug encapsulation and delivery. Vector selection and recombination form a critical step in microbial decoration methodologies. By selecting the appropriate vector and undertaking recombination operations, genomic and surface attributes of microorganisms, such as bacteria, can be regulated [63]. The precise selection strategy and recombination plan are crucial in accomplishing efficient, precise, and controlled microbial decorations. This section will delve into the factors related to vector selection and recombination, and provide insight into application cases across various domains.

(1) Vector selection

Plasmids are commonly used vectors for gene transmission and expression that are suited for introducing exogenous genes into or editing genes in microorganisms [64]. Various types of plasmids exist, each with different copy numbers, replication mechanisms, and selection markers, thus necessitating selection based on experimental requirements [65]. Neves et al. [66] studied and probed the condensation ability of RALA peptide on p53 encoding plasmid DNA (pDNA) to generate a suitable intracellular delivery platform. These vectors were formed in varying nitrogen-to-phosphorus (N:P) ratios based on shape, size, surface charge, payload, and complexation capabilities characterized. The fine structures were assessed via Fourier-transform infrared (FTIR) spectroscopy. Confocal microscopy studies confirmed the intracellular localization of nanoparticles, hence improving continuous pDNA uptake. Additionally, *in vitro* transfection with RALA/pDNA vector-mediated HeLa cells facilitated gene release and expression of p53 protein. Apoptosis in cancer cells was studied based on these advances. The N:P ratio effectively regulates gene transfection efficiency. Therefore, the N:P ratio could be fine-tuned based on the required protein expression and apoptosis level. The tremendous asset of this system stems precisely from the utilization of the N:P ratio as a tailoring parameter, which can modify not only vector characteristics but also adjust the degree of pDNA delivery and protein expression, thereby modulating the therapeutic efficacy of p53 mediated cancer treatment.

(2) Viral vectors

Viral vectors serve as an effective gene delivery tool for efficiently introducing exogenous genes into target microorganisms [67]. Chan et al. [68] reported a strategy utilizing short DNA oligonucleotides to antagonize TLR9 activation to decrease the immunogenicity of the adeno-associated virus (AAV) vector. Direct insertion of TLR9 antagonists into the AAV vector genome resulted in an engineered vector that significantly lowered innate immunity and T-cell responses triggered in clinically relevant mouse and pig models, thereby enhancing gene expression in various tissues, including the liver, muscles, and retina. Compared to non-engineered vectors, engineered vectors were capable of "disguising" the vector and preventing the emergence of unnecessary immune responses. This "coupled immune modulation" strategy potentially broadens the therapeutic window for AAV therapy as well other gene transfer methods. The report mentioned the discovery of TLR9 antagonists. TLR9 antagonists are short-chain DNA oligonucleotides capable of binding TLR9 to block its activation. Previous studies have shown that TLR9 antagonists alone reduce immune responses prompted by AAV vectors in the liver and target cells. However, this strategy requires high quantities of oligonucleotides to suppress all TLR9 molecules, which might be impractical in the clinical setting. Consequently, this study proposed direct insertion of TLR9 antagonists into the AAV vector genome to decrease AAV immunogenicity. In summary, the above research successfully reduced AAV immunogenicity by introducing TLR9 antagonists into the AAV vector, widening the therapeutic window of AAV therapy and other DNA gene transfer methods.

(3) Integrated vectors

Integrating vectors have the ability to insert exogenous genes into the microbe genome, thereby making the decoration results more stable and long-lasting. Commonly used integrated vectors include integrative plasmids and viruses. Urello et al. [69] demonstrated a novel method that stably incorporates plasmids (DNA) into collagen scaffolds, thus capitalizing on the natural process of collagen reconstruction to efficiently achieve non-viral gene delivery. CMPs binding with DNA polyplexes, along with the inherent affinity between CMPs and collagen proteins, not only improved the control over polyplex retention and release, but also a range of substantial and highly unique benefits were provided through the stable and enduring link between CMP polyplexes and collagen protein fragments. In conclusion, these findings showcased significant improvement with CMP decoration in terms of gene retention, altering release dynamics, serum stability, and *in vivo* gene activity. This versatile technique has enormous potential for various applications in regenerative medicine.

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(4) Gene decoration

Improvements to microbe traits and functions are achieved by altering the sequence of target genes, such as point mutations or by inserting or deleting specific fragments. Sauer et al. [70] used a precise gene-editing technology known as oligonucleotide-directed mutagenesis (ODM). This technique utilizes chemically synthesized oligonucleotides as repair templates which, by homologous pairing with target site DNA, allows for precise editing of specific sites within the genome. The method has been successfully used in several systems, including bacteria, yeast, mammals, and plants. Additionally, microbial traits and functions can be modified by introducing exogenous genes into specific locations within the microbial genome using specific endonucleases and recombination enzyme systems [71, 72]. This process allows for targeted manipulation of the microbe genetic material, enabling the introduction of new traits and the enhancement of desired functions. By carefully selecting the target sites and utilizing precise gene insertion methods, researchers can effectively engineer microbes for various applications, such as bioremediation, biofuel production, and pharmaceutical development [73]. Alteration of microbe traits and functions is achieved by inserting exogenous genes at specific sites in the microbe genome [74, 75]. Commonly used strategies include insertion mediated by specific endonucleases and recombination enzyme systems.

Selection of carrier vectors and recombination are key steps in microbial decoration methods. By choosing suitable vectors and flexible recombination strategies, regulation of the microbe genome and surface properties can be achieved. Plasmid, viral, and integrated vectors are commonly used vector options, while gene insertion, gene deletion, and gene decoration are common recombination strategies [76, 77]. Through the correct selection of vectors and flexible use of recombination strategies, efficient, accurate, and controllable microbial decoration offers new possibilities for drug delivery and bio-production.

3. DRUG DELIVERY AND TARGETING APPLICATIONS IN DISEASE TREATMENT

Microbial decoration has wide applications in drug delivery. Precise tumor therapy is achieved by modifying the anti-tumor drug carrier on the bacterial surface, enabling the bacteria to specifically accumulate in tumor tissues [78, 79]. With improvement in traditional drug delivery systems, the use of microbial decorations improve the stability of drug molecules, control the release rate, and increase the efficiency of drug delivery. The effectiveness of microbial modification in addressing key challenges in drug delivery is evident.

(1) Targeted drug delivery

Microbial components, such as bacterial cell membranes or viral envelopes, can be utilized to cloak drug carriers. This camouflage allows for improved evasion of the immune system and targeted delivery to specific tissues or cells. For example, liposomes coated with bacterial membrane fragments have demonstrated enhanced targeting to infected tissues, optimizing the therapeutic effect while minimizing off-target effects. To overcome the limitations of curcumin, a multi-targeting pharmacologically active compound, in the treatment of skin inflammation and infection in chronic wounds Ternullo et al. [80] prepared deformable liposomes (DLs) containing curcumin with varying surface charges, including neutral (NDLs), cationic (CDLs), and anionic (ADLs). Ternullo et al. [80] explored the properties and biological effects of these curcumin-containing DLs (curcumin-DLs). All DLs significantly inhibited the growth of *Staphylococcus aureus* and *Streptococcus pyogenes in vitro*. The incorporation of curcumin into DLs not only achieved sustained skin penetration but also enhanced its biological properties.

(2) Stabilization of drug delivery vehicles

Incorporating microbial elements, such as proteins or polysaccharides derived from bacteria or fungi, enhance the stability of drug carriers. These components provide structural integrity and protect against premature drug leakage or degradation. For example, the use of bacterial exopolysaccharides in nanoparticle formulations has been shown to improve the stability of the carriers during storage and transportation, ensuring the integrity of the drug payload. Shi et al. [81] developed a novel capsular polysaccharide (CPS) nanoparticle (CPS-am NPs) by utilizing a naturally occurring capsular polysaccharide from *Lactobacillus plantarum* LCC-605 to encapsulate amikacin. These nanoparticles demonstrated improved and sustained antibacterial and antibiofilm activities against *Escherichia coli* and *Pseudomonas aeruginosa* [81].

(3) Controlled release

Microbial decoration can also be harnessed to achieve controlled release of drugs. By leveraging the natural stimuli-responsive characteristics of some microorganisms, drug release can be modulated in response to specific environmental cues. An illustrative example is the use of genetically engineered bacteria that respond to pathologic conditions, triggering the release of therapeutic agents at the target site. Du et al. [82] utilized drug-loaded nanoparticles in combination with genetically engineered bacteria to achieve targeted therapy in tumor hypoxic areas. Using cationic lipid nanoparticles (PTX-cls) as the drug carriers, Du et al. [82] coupled the PTX-cls with gas vesicle-carrying transgenic *Escherichia coli* (GVs-E) to specifically target tumors and enhance high-intensity

focused ultrasound (HIFU) ablation. This combined nanosystem enabled both chemotherapy and synergistic HIFU treatment of tumors.

3.1 Bacterial decoration to treat tumors

Bacteria have been modified into drug carriers to achieve targeted tumor treatment through selective accumulation in tumor tissue. For example, using modified *Salmonella* as a drug carrier, carrying anti-tumor genes or drugs, *Salmonella* can deposit and release drugs in tumor tissues to achieve the effect of tumor treatment [83]. In recent years, the incorporation of bacterial decoration into functional nanoparticles has achieved precise antitumor effects on tumors through a therapeutic response, especially the Fenton-like response. For example, Fan et al. [84] designed an engineered bacterium (*E. coli* MG1655) with NDH-2 enzyme (respiratory chain-enzyme II) overexpression (Ec-pE) to colonize the tumor region and increase local H_2O_2 generation, as shown in **Figure 4**. On this basis, magnetic Fe_3O_4 nanoparticles were covalently linked to bacteria as a catalyst for the Fenton reaction to convert H_2O_2 into a toxic hydroxyl radical (OH) for tumor therapy. In the constructed bioreactor, the engineered bacteria continuously synthesize H_2O_2 undergoing Fenton-like reactions and induce severe tumor apoptosis through the resulting toxic OH.

By combining immunotherapy drugs with microbial decorations, the effectiveness of immunotherapy can be enhanced. For example, modified bacteria can carry antigens and enhance antigen-specific cellular immune responses by stimulating the immune system, thereby improving the effectiveness of cancer. Nguyen et al. [85] proposed a novel tumor therapy bacterial microrobot (bacteriobot) that combines paclitaxel-loaded liposomal microcargo with tumor-targeted *Salmonella typhimurium*. As shown in **Figure 5**, the tumor therapeutic liposome bacterial robots were constructed by combining biotin molecules displayed on bacterial outer membrane proteins with streptavid wrapped in drug-loaded immunotherapy liposomes. Bacterial robots exhibit powerful tumor targeting and killing properties, which can be used for the active treatment of tumors.

3.2 Viral-modifying treatment of genetically-defective diseases

In diseases characterized by genetic deficiencies, the body's ability to produce proteins or function properly is compromised due to gene mutations or deletions, resulting in severe conditions, such as hereditary immunodeficiency disease and cystic fibrosis [86, 87]. It difficult for conventional therapies to directly repair these genetic defects, so virus-modifying therapy has become a potential option. The rationale of virus-modifying therapy is the use of the modified virus as a gene delivery tool. These viruses are genetically engineered to remove pathogenic genes and carry normal gene sequences. When these viruses enter the patient, the viruses infect the target cells and release DNA or RNA containing the normal gene. These normal genes will be utilized by the cells and produce the desired proteins in a normal manner, thus correcting the gene defect.

Commonly used viral vectors include adeno-associated viruses (adenoviruses), adenovirus-associated viruses (AAVs), and HIV-like viruses (lentiviruses), which are widely used in the field of genetic engineering. These viruses can efficiently deliver genes to target cells and exist stably *in vivo* for a period of time, thus providing lasting efficacy. For example, Donadon et al. [88] used the virus as a vector to insert genetic decorations into the genome, then used the viruses for treating diseases caused by genetic defects. For example, a modified AAV can carry a normal gene and inject the normal gene into the affected

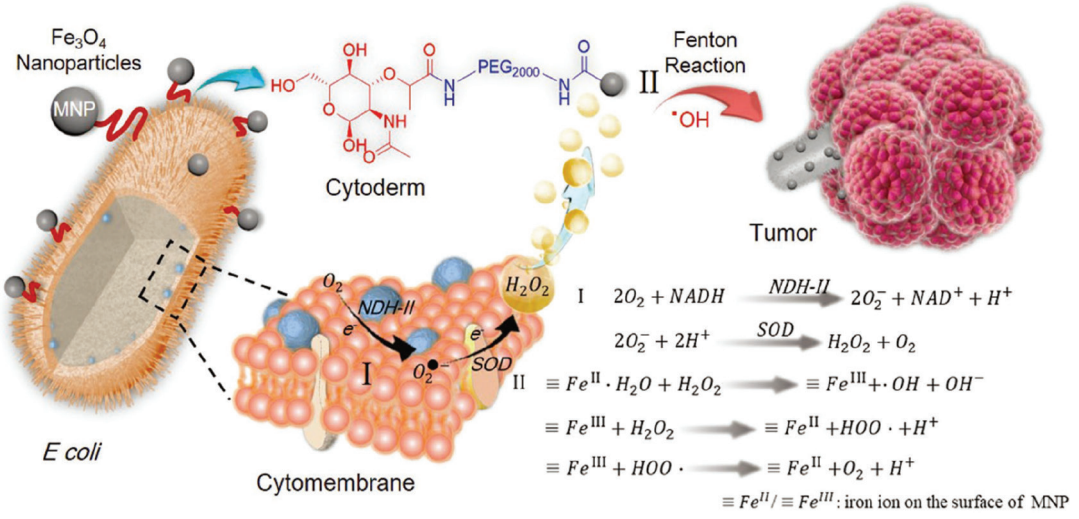

Figure 4 | The scheme of bacteria-based Fenton-like bioreactor and its chemodynamic therapy process for antitumor therapy [84].

Acta Materia Medica

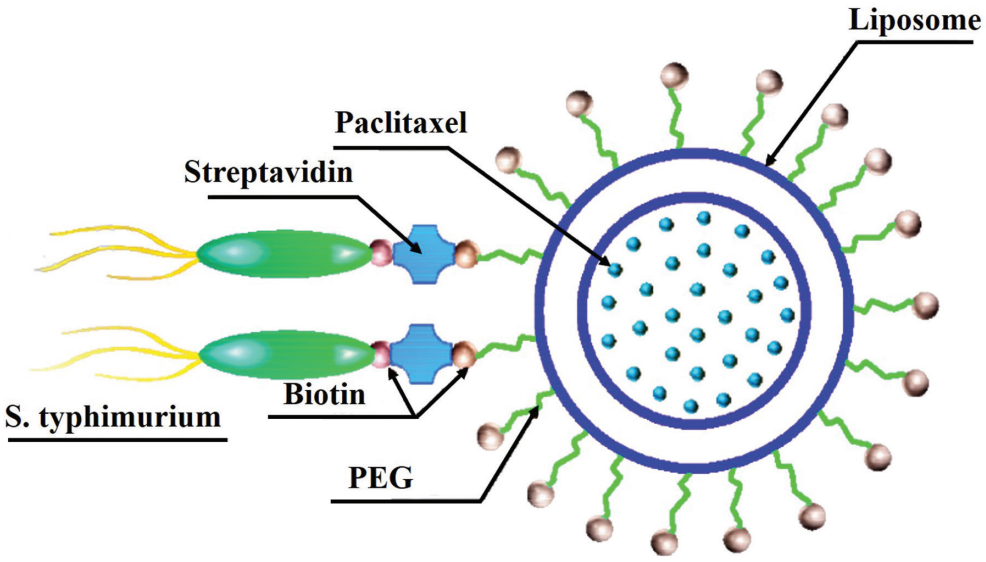

Figure 5 | Schematic diagram of a therapeutic liposomal bacteria-based microrobot (DL bacteriobot) [85].

cells to treat spinal muscular atrophy. This therapy may also bring another potential benefit to patients. Because the genetic correction by virus-modifying therapy occurs at the cellular level, the corrected genes post-treatment may persist within the patient and continue to generate the required proteins to provide a sustained therapeutic effect. This enduring correction effect makes virus-modifying therapy exceptionally attractive in the treatment of genetic deficiency diseases, offering patients a novel and effective treatment option.

3.3 Antibiotic delivery

Antibiotic resistance is a global health problem and a current threat to modern medicine and society. Bacterial delivery antibiotic systems are methods that use bacteria with natural localization and invasive capabilities as carriers to deliver antibiotics to specific infection sites to improve antibiotic efficacy and reduce side effects [89]. Bacterial resistance to antibiotics can occur through multiple mechanisms. These mechanisms include overexpression of antibiotic target enzymes and proteins, mutations in antibiotic targets and enzymes, overexpression of antibiotic-modifying enzymes, such as β-lactamases, and the use of multiple efflux pumps to actively export antibiotics to bacterial cells, therefore reducing the accumulation of intracellular antibiotics [90]. New strategies for antibiotic drug design and delivery offer a glimmer of hope for the currently limited new antibiotic pipeline. One strategy is to conjugate iron-chelating microbe siderophores to antibiotics or antimicrobial agents to enhance absorption and antimicrobial potency. Using microbial decoration, the antibiotics are fixed on the microbe surface or embedded in the microorganism and the directional sterilization of infectious pathogens through the directional transport and release of microorganisms is realized. This strategy could improve antibiotic efficacy and reduce resistance to antibiotics.

More and more bacterial species have shown inherent tumor targeting characteristics. Shi et al. [91] designed a *Salmonella typhimurium* strain (ST8). ST8/pSEndo carrying therapeutic plasmid-encoding endostatin and secretory protein SopA fusion can target tumor vessels, and stably maintain and safely deliver the treatment carrier through type III secretion system (T3SS) release of angiogenesis inhibitors, thereby interfering with the angiogenic effect of growth factors in tumors. Mouse CT26 colon cancer mice injected with ST8/pSEndo showed effective tumor suppression by inducing more severe necrosis and inhibiting the vascular density within the tumor.

3.4 Treatment of other diseases

Metabolic disorders, including obesity, diabetes, and cardiovascular disease, are prevalent in westernized countries. Gut microbiota composition is a contributing factor to an individual's susceptibility to the development of these diseases. Therefore, changing a person's microbiome may improve such diseases [92, 93]. One potential strategy for microbiome change is the combination of modified bacteria expressing therapeutic factors into the gut microbiota. For example, Chen et al. [94] incorporated transgenic bacteria secreted N-acylphosphatidylethanolamines (ape) into the gut microbiota to provide continuous treatment for chronic diseases and obesity, thus reducing the need for daily continuous administration of therapeutic compounds. Cardiovascular disease (CVD) is a major cause of morbidity worldwide and is influenced by genetic and environmental factors. Recent advances have provided scientific evidence that CVD may also be attributed to the gut

microbiota [95]. Microbial decoration can be used in the treatment of CVDs. For example, modified lactic acid bacteria can carry drug genes with lipid-lowering and anti-platelet aggregation activities and be administered orally to patients, thus improving blood lipid metabolism and preventing the occurrence of CVD.

These application cases demonstrate the diversity and potential of microbial decoration in drug delivery. As an innovative delivery strategy, microbial decoration provides new ideas and methods for precision therapy and improving drug efficacy. Although there are still some challenges in the application process, such as safety, stability, and precision of control release, progress in technology and further research is ongoing. Microbial decoration is expected to exert greater potential in drug delivery and bring new breakthroughs in disease treatment.

Furthermore, the connections and potential opportunities for collaboration between microbial decoration technology and other fields, such as nanotechnology, biomaterials, and pharmacology, are indeed crucial to advancing this innovative drug delivery strategy. Here are some relevant literature and examples that support the feasibility and value of interdisciplinary research: (1) Nanotechnology offers unique tools and techniques for engineering nanoparticles that can be integrated with microbial surfaces to enhance drug delivery. For example, Wang et al. [93] explored the use of nanocarriers coated with microbial decoys to improve drug targeting and release efficiency. (2) Biomaterials have a crucial role in designing drug delivery systems and their integration with microbial surfaces can further enhance their functionality. For example, incorporating biocompatible polymers into microbial decoration techniques can improve the stability and biocompatibility of drug carriers [96]. (3) Collaborations between pharmacology and microbial decoration can deepen our understanding of drug interactions, optimize drug loading strategies, and enhance therapeutic outcomes. By combining expertise from both fields, researchers can develop more effective targeted drug delivery systems [97].

Overall, interdisciplinary research involving nanotechnology, biomaterials, and pharmacology can synergistically contribute to advancing microbial decoration-based drug delivery systems. By leveraging the expertise and tools from these fields, researchers can overcome technical challenges, develop smarter carrier materials, improve targeting efficiency, and ultimately enhance the translation of microbial decoration technology into clinical applications.

4. CONCLUSIONS AND FUTURE PERSPECTIVES

Microbial decoration has a broad application prospect as an innovative drug delivery strategy. Microbial decoration methods offer unique advantages in addressing challenges faced by existing drug delivery systems. By modifying microbial surfaces, drug delivery vehicles can overcome limitations, such as poor drug bioavailability, limited targeting, and rapid drug degradation. This approach enhances stability, improves targeting specificity, and controls drug release, ultimately increasing drug efficacy. Additionally, microbial decoration enables site-specific drug delivery, minimizing off-target effects, reducing systemic toxicity, and improving therapeutic outcomes. Microbial decoration also allows for the simultaneous delivery of multiple drugs, enabling combination therapies to enhance treatment efficacy. Personalized medicine can be achieved by tailoring microbial decoration techniques to individual patient profiles. Collaborations with other disciplines, such as nanotechnology and pharmacology, can drive further advancements in this field. Safety and biocompatibility assessments are crucial to ensure the effectiveness and safety of microbial decoration-based drug delivery systems. Overall, emphasizing the potential of microbial decoration methods and promoting continued research and collaboration will propel this innovative drug delivery strategy forward. By altering the properties and functions of microbe surfaces, microbial decoration can improve the specific delivery of drugs, enhance the stability of drug delivery vehicles, and control release, thereby improving the efficacy of drug therapy and reducing side effects.

With the continuous development of microbial decoration methods, we can expect more breakthroughs and innovations in the field of drug delivery. In the future, scientists can further optimize microbial decoration techniques to improve the specificity and efficiency of drug delivery. In addition, exploring new decoration methods and carrier materials can make the drug delivery system smarter and more precise. Strengthening cross-research with nanotechnology, biomaterials, and other fields will help develop more advanced drug delivery technologies. Moreover, compared to traditional drugs, microbial decoration-based drug delivery systems pose certain technical and safety challenges that require further research and addressing. Therefore, future research directions also include exploring new decoration methods, gaining insights into molecular interaction mechanisms during decoration, and evaluating the biocompatibility and safety of modified systems in individuals and the environment. In conclusion, microbial decoration, as a promising drug delivery strategy, will continue to receive attention and research to provide more precise and efficient methods for clinical treatment.

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CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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