International Journal of Nanomedicine

Dovepress
Taylor & Francis Group

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REVIEW

Nanomaterials: A Prospective Strategy for Biofilm-Forming *Helicobacter pylori* Treatment

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Abstract: *Helicobacter pylori* (*H. pylori*) is prevalent in over 50% of the global population and is recognized as the primary etiological agent for the development of gastric cancer. With the increasing incidence of antibiotic resistance, clinical treatment of *H. pylori* is a significant challenge. The formation of *H. pylori* biofilm is an important reason for antibiotic resistance and chronic infection, and it is also one of the key obstacles to eradicating *H. pylori. H. pylori* biofilm acts as a physical barrier, preventing the penetration of antibiotics and increasing the expression of efflux pump genes and drug-resistant gene mutations. Therefore, the treatment of *H. pylori* biofilm is extremely challenging. Nanomaterials, such as inorganic nanoparticles, lipid-based nanoparticles, and polymeric nanoparticles, which have properties including disrupting bacterial cell membranes, controlling drug release, and overcoming antibiotic resistance, have attracted significant interest. Furthermore, nanomaterials have the ability to treat *H. pylori* biofilm owing to their unique size, structure, and physical properties, including the inhibition of biofilm formation, enhancement of biofilm permeability, and disruption of mature biofilm. Moreover, nanomaterials have targeting functions and can carry antimicrobial drugs that play a synergistic role, thus providing a prospective strategy for treating *H. pylori* biofilm. In this review, we summarize the formation and antibiotic-resistance mechanisms of *H. pylori* biofilm and outline the latest progress in nanomaterials against *H. pylori* biofilm with the aim of laying the foundation for the development and clinical application of nanomaterials for anti–*H. pylori* biofilm.
Keywords: nanomaterials, *H. pylori*, biofilm, treatment, antibiotic resistance, prospective strategy

Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative, spiral-shaped, microaerophilic bacterium that specifically colonizes the human stomach of around 50% of the global population, with a higher prevalence in developing countries.[1–4] *H. pylori* is a significant contributor to a broad range of gastrointestinal diseases, including chronic gastritis, peptic ulcer disease, autoimmune diseases, and gastric adenocarcinoma.[5,6] In 1994, *H. pylori* was classified as a class I carcinogen by the World Health Organization.[7] By the end of 2021, the United States had updated its 15th report on carcinogens, adding eight new carcinogenic substances and listing *H. pylori* as a definite carcinogen.[8] In addition, increasing research suggests that chronic infection with *H. pylori* is associated with a series of extraintestinal diseases, such as coronary heart disease, iron-deficiency anemia, and neurological disorders.[9–11] Hence, the eradication of *H. pylori* is necessary for the maintenance of human health.

The current treatment of *H. pylori* mainly relies on antibiotics. However, with the widespread use of antibiotics, the problem of bacterial resistance has become increasingly prominent.[12,13] Among the various antibiotic-resistance mechanisms of *H. pylori*, the formation of bacterial biofilm is an important factor.[14] As a bacterial barrier, biofilm reduces or delays the entry or penetration of antibiotics, preventing them from contacting the bacterial cells, thereby leading to antibiotic resistance and causing persistent and chronic *H. pylori* infections in the host.[15,16] Moreover, efflux pump genes that form biofilm are highly expressed in *H. pylori*, promoting the development of antibiotic resistance.[17] Furthermore,

Received: 14 December 2024
Accepted: 7 April 2025
Published: 23 April 2025

the morphology of *H. pylori* within biofilm changes from spiral to coccoid form, leading to increased tolerance to antibiotics.[18] Therefore, when conventional antibiotics are prescribed clinically, their efficacy in eradicating biofilm-associated *H. pylori* infection is significantly reduced due to the aforementioned factors. Alternative therapies for *H. pylori* biofilm have been explored. For example, antimicrobial peptides possess broad-spectrum antibacterial activity and can penetrate the extracellular matrix of biofilm to directly target and disrupt bacterial cells.[19–21] Studies have shown that the antimicrobial peptides DJK-5 and IDR-1018 can inhibit the growth and maturation of *H. pylori* biofilm.[22] Some probiotics can also interfere with *H. pylori* biofilm. For instance, *Lactobacillus fermentum* UCO-979C and *Lactobacillus plantarum* LN66 can inhibit the formation of *H. pylori* biofilm.[23,24] Moreover, certain natural products can disrupt *H. pylori* biofilm. Silvia et al used *Pistacia vera* L. oleoresin in combination with levofloxacin to synergistically disrupt *H. pylori* biofilm.[25] However, these studies are still relatively immature, and the efficiency of antibiofilm activity as well as biosafety needs to be improved. Therefore, it is important to develop innovative treatment approaches that target *H. pylori* biofilm.

With the rapid development of nanotechnology, an increasing number of nanomaterials have shown great potential in the field of biofilm treatment.[26–28] Nanomaterials consist of particles with dimensions ≤100 nm.[29] Depending on the matrix components, nanomaterials used for antibacterial purposes can be classified into various types, such as inorganic nanoparticles (NPs; including metal-based NPs or their oxides), lipid-based NPs (eg, liposomes, nanoemulsions), and polymer NPs (natural or synthetic polymers).[30] Nanomaterials have several benefits, including diminutive size, elevated specific surface area, substantial drug-carrying potential, and the ability to release drugs in response to specific stimuli. These characteristics enable them to overcome current resistance barriers and enhance the efficacy of biofilm eradication.[31–33] Some studies on these nanomaterials have already entered the clinical trial stage. For instance, amikacin liposomes have been used to treat pulmonary infections caused by *M. tuberculosis* in biofilm.[34] In addition, nanomaterials have been used to treat *H. pylori* biofilm at different stages. First, lipid–polymer NPs (LPNs) can interfere with the attachment of *H. pylori* to the gastric epithelium, thereby blocking biofilm formation.[35] Polymer NPs can enhance the permeability of *H. pylori* biofilm by adjusting their surface properties.[36] Inorganic NP-based photothermal therapy/photodynamic therapy can directly destroy mature *H. pylori* biofilm by interacting with the biofilm through oxidative stress and hyperthermia.[37] Moreover, an increasing number of studies are focused on drug-delivery nanocarriers that have been engineered to specifically target *H. pylori* biofilm and regulate the release of antimicrobial agents, aiming to reduce the usage of antibiotics and decrease adverse side effects.[38,39]

In this review, we first provide a brief introduction to the biological traits of *H. pylori* biofilm and highlight its fundamental mechanisms of formation. We review current studies on the mechanisms of *H. pylori* biofilm–enhancing antibiotic resistance. Furthermore, we discuss advancements in research regarding the interaction of various nanomaterials with *H. pylori*, focusing on their biofilm-targeting capabilities and summarizing their properties and mechanisms of action against *H. pylori* biofilm. Finally, we assess the prospects and challenges associated with the application of nanotechnology in the treatment of *H. pylori* biofilm.

Formation of *H. pylori* Biofilm

Key Features of *H. pylori* Biofilm

A biofilm is a microbial community that embeds bacteria with an extracellular polymeric substance (EPS) matrix, which can cause persistent infectious diseases and pose significant challenges to anti-infection therapy.[40–42] Researchers have indicated that *H. pylori* can form biofilm in both vitro (Figure 1A and B) and vivo (Figure 1C and D) settings.[43–45] Biofilm provides an environment that is most conducive to the survival of *H. pylori* in harsh conditions, including extreme acidity, nutrient and oxygen deficiency, and the presence of antibiotic.[46] The EPS is an important component of *H. pylori* biofilm, and is primarily composed of polysaccharides, extracellular proteins, extracellular DNA (eDNA), and outer-membrane vesicles (OMVs).[22,47]

Polysaccharides, including glucose, *N*-acetylglucosamine, fucose, and galactose, are ideal molecules for promoting bacterial adhesion and forming the three-dimensional scaffold of biofilm.[29,48–50] Proteins contribute to the stabilization of the matrix network formed by polysaccharides and enhance the binding between bacteria.[51] Research has shown that

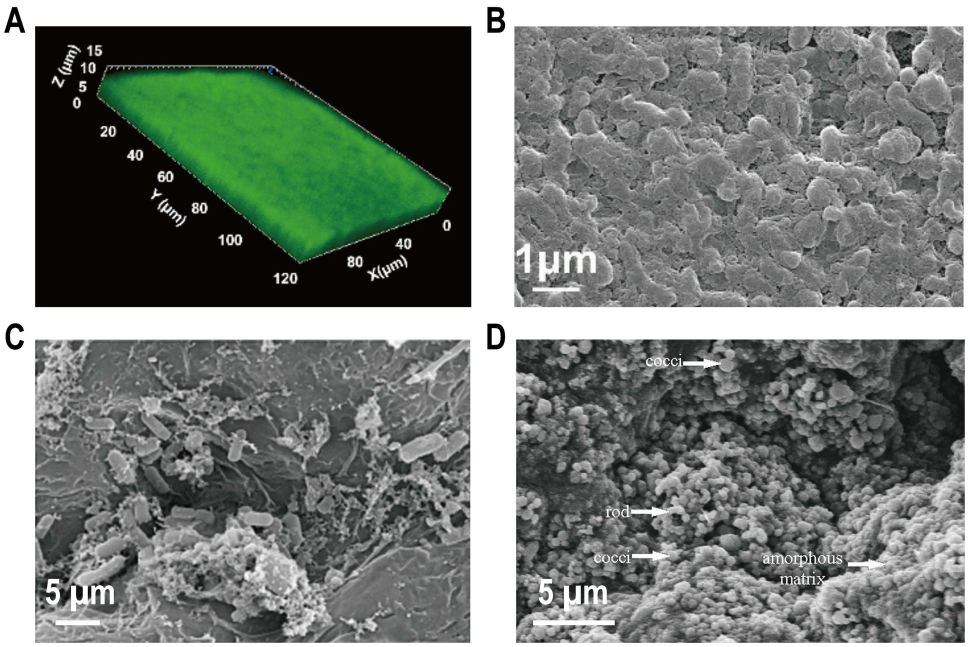

Figure 1 *H. pylori* biofilm formation in vitro and in vivo. (**A**) Confocal laser-scanning microscopy (CLSM) image of *H. pylori* standard strain 26695 biofilm in vitro. Membrane-permeant SYTO 9 (green) was used to stain bacterial cells. Reproduced from Zhao Y, Chen Z, Cai Y, et al. Aloe-emodin destroys the biofilm of *Helicobacter pylori* by targeting the outer membrane protein 6. Microbiol Res. 2024;278:127539. Copyright (2024), with permission from Elsevier.[43] (**B**) Scanning electron microscopy (SEM) image of *H. pylori* standard strain 26695 mature biofilm grown on a nitrocellulose membrane for 3 days. Reproduced from Zhao Y, Chen Z, Cai Y, et al. Aloe-emodin destroys the biofilm of *Helicobacter pylori* by targeting the outer membrane protein 6. Microbiol Res. 2024;278:127539. Copyright (2024), with permission from Elsevie.[43] (**C**) SEM image of gastric tissue in mouse model infected with *H. pylori*. Reproduced from Cheng X, Geng J, Wang L, et al. Berberine-loaded mannosylerythritol lipid-B nanomicelles as drug delivery carriers for the treatment of *Helicobacter pylori* biofilms in vivo. Eur J Pharm Biopharm. 2023. Copyright (2023), with permission from Elsevie.[44] (**D**) SEM image of gastric tissue from a patient infected with urease-positive *H. pylori*. Reproduced from Carron MA, Tran VR, Sugawa C, et al. Identification of *Helicobacter pylori* biofilms in human gastric mucosa. J Gastrointest Surg. 2006;10(5):712–717. Copyright (2006), with permission from Elsevie.[45]

proteins are essential for the EPS within *H. pylori* biofilm and that protease treatment of the biofilm significantly leads to its dispersion.[16,22] The presence of eDNA within the biofilm matrix of various bacterial species has been identified, and it is believed to contribute to the biofilm's structure, facilitate genetic exchange, and influence bacterial diversity.[52,53] Nevertheless, the function of eDNA within *H. pylori* biofilm remains to be comprehensively elucidated,[54] but research suggests it may be related to the aggregation of OMVs.[55] OMVs are double-layered structures with diameters ranging from 20 to 500 nm, and contain proteins, phospholipids, DNA, and lipopolysaccharides.[56] OMVs have been demonstrated to be a part of the biofilm matrix in some Gram-negative bacteria, including *H. pylori*.[57,58] Additionally, OMVs were observed in the biofilm of *H. pylori* strain TK1402, which forms strong biofilm, whereas no OMVs were detected in strains that form weak biofilm. This suggests that OMVs may promote the formation of *H. pylori* biofilm.[58,59] These EPS components could act as potential targets for designing nanomaterial-based antibacterial agents to treat *H. pylori* biofilm.

Mechanisms of *H. pylori* Biofilm Formation

As with many other bacterial species, *H. pylori* biofilm formation is a complex, multistage process that encompasses bacterial adhesion, biofilm assembly, maturation, and eventual dispersion (Figure 2).[60] In the following sections, we analyze in detail the characteristics and mechanisms of each step in the growth of *H. pylori* biofilm.

Adherence

Adhesion is the primary step in the formation of *H. pylori* biofilm, and its role is maintained throughout the entire biofilm-development process.[61,62] Research has found that *H. pylori* can adhere not only to gastric epithelial cells but also to nonbiological surfaces.[63–65] It is interesting that the initial adhesion of *H. pylori* is negatively correlated with the concentration of fetal bovine serum (FBS) added, though FBS typically promotes the growth of planktonic bacteria.[66] Some studies have also found that the adherence of *H. pylori* to gastric epithelial cell surfaces does not depend on the presence of FBS, indicating that *H. pylori* may utilize a special surface-attachment mechanism.[67,68] *H. pylori* can also

https://doi.org/10.2147/IJN.S512066

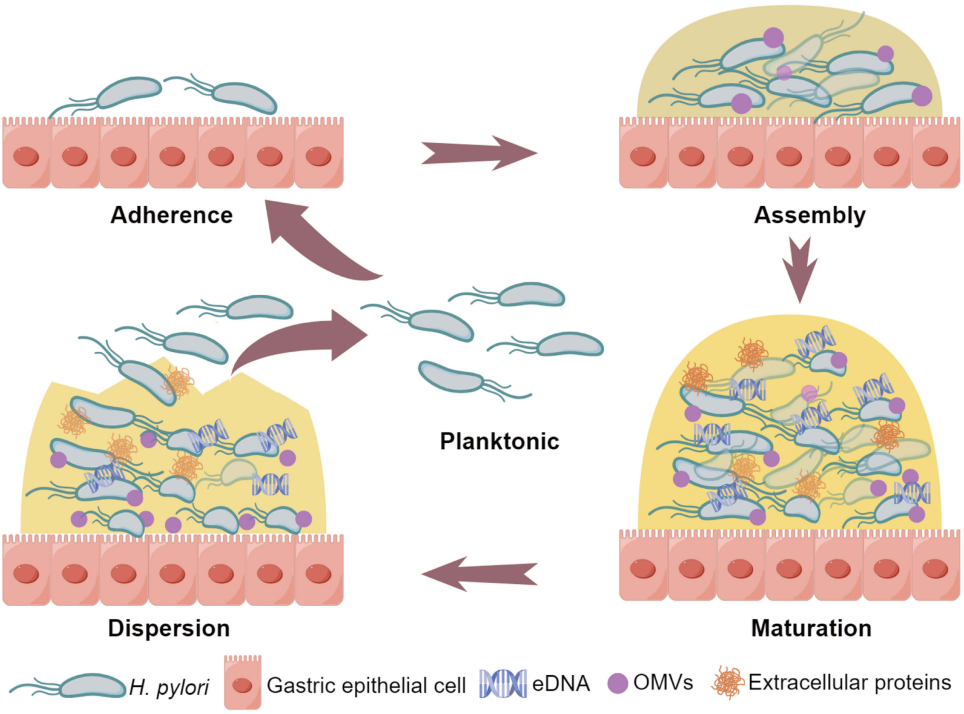

Figure 2 Schematic representation of *H. pylori* biofilm-formation mechanism. Created by FigDraw.

adhere to various types of nonbiological materials, such as the inner surfaces of drinking-water system pipelines, and then form biofilm.[63,69] Different strains of *H. pylori* also exhibit varying adhesion capabilities, and this variation may be due to the heterogeneity caused by different outer-membrane proteins, regulatory proteins, and flagella in different *H. pylori* strains.[62,65,70]

Assembly

After surface adhesion, *H. pylori* upregulates the secretion of signaling molecules between bacteria on the adherent surface through cooperative and altruistic behaviors, collectively producing EPS with the surrounding *H. pylori* and gradually forming structurally complex microcolonies.[64,71,72] Research has found that different *H. pylori* strains can form multicellular aggregates within a few hours in vitro, with the development of more intricate structures over the course of a day's incubation.[73] Research on the *H. pylori* strain G27 observed that biofilm was first assembled at the liquid–air interface after 20 hours, followed by the assembly of aggregates both at and below the interface as the biofilm developed, with the distribution of EPS being consistent with this growth trend.[22] Scanning electron microscopy has elucidated that flagella are crucial for the structural integrity of *H. pylori* biofilm, with flagella-less *H. pylori* assembling biofilm slowly.[61] Comparative genomics studies further indicate that the assembly speed of *H. pylori* biofilm is similar among different strains.[62] In addition, research has shown that the assembly of *H. pylori* biofilm is largely unaffected by in vitro factors, such as continuous passage and nutritional components.[22]

Maturation

Mature *H. pylori* biofilm undergoes notable morphological changes, coincident with a marked increase in EPS synthesis.[74] The maturation process of *H. pylori* biofilm is intricately linked to the formation of EPS, which is vital for biofilm development.[16] Investigations have indicated that the maximum amount of *H. pylori* biofilm is typically observed at the 3-day mark in vitro and that it can persist for up to a week under various culture conditions, indicating that the biofilm-maturation process is time-dependent.[16,61,65,75,76] Observations through SEM have revealed a special feature of *H. pylori* biofilm, flagellar filaments, which have been found to promote surface cohesion and intercellular connections, and together with the formation of filaments maintain the integrity of the *H. pylori* biofilm structure on biological and

nonbiological surfaces.[16,64] Research has indicated that *H. pylori* mutants deficient in flagella production (Δ*fliM*, Δ*fliA*) have diminished capacity to develop biofilm, which is partly due to the decrease in flagellar filaments within the biofilm structure.[16] *H. pylori* biofilm formation involves the conversion of flagellar structures into adhesive properties, which further elucidates the significance of flagella in the biofilm's growth and maturation process.[64] In mature *H. pylori* biofilm grown on nonbiological surfaces, a majority of *H. pylori* is in coccoid form, with a minority being rod-shaped.[64] The coccoid morphology of *H. pylori*, once considered a stress-induced response, has mechanisms that remain largely unexplored and not completely understood.[77] Recently, research has shown that coccoid *H. pylori* maintains its membrane integrity and metabolism during incubation periods as long as 70 hours, suggesting that it is viable and in a dormant state.[78] An analysis of the morphological structure of *H. pylori* biofilm indicated that clarithromycin (CLR)-resistant *H. pylori* strains form thicker biofilm with higher coccoid-morphology prevalence.[74]

Dispersion

Like biofilm of other bacteria, *H. pylori* biofilm disperses once it has reached its optimal growth and depleted its nutrients, as evidenced by a reduction in crystal violet staining.[16,64,79] There is relatively little research on the signaling mechanisms of *H. pylori* biofilm dispersion, yet some studies indicate that *H. pylori* uses the quorum-sensing molecule AI-2 to modulate both biofilm formation and dispersion.[72] The quorum-sensing molecule AI-2 can be expressed by *H. pylori* through the *luxS* gene, which is bacterial density–dependent, indicating that *H. pylori* can regulate its local density via the secretion of AI-2.[80] Additionally, research has demonstrated that AI-2 promotes the dispersion of *H. pylori* biofilm and an absence of the *luxS* gene (Δ*luxS*) leads to a significant increase in biofilm formation compared to the wild-type *H. pylori* strain.[72] Furthermore, it is believed that the chemotaxis system promotes the dispersion of *H. pylori* biofilm by detecting and reacting to AI-2 molecules, as the chemotaxis histidine kinase mutant Δ*cheA* exhibits a biofilm phenotype similar to that of the Δ*luxS* mutant.[72] The mechanisms by which *H. pylori* regulates biofilm maturation and dispersion are not fully understood and require further investigation.

Mechanism of *H. pylori* Biofilm Enhancing Antibiotic Resistance

Antibiotic resistance refers to an increase in the minimum inhibitory concentration of antibiotics that results from enduring alterations within bacteria, including mutations in resistance genes or the acquisition of resistance through horizontal gene transfer.[81] Compared to planktonic *H. pylori*, its biofilm state increases adaptive resistance to antibiotics 10–1000-fold.[82,83] However, the resistance mechanisms of *H. pylori* biofilm have not been fully elucidated, leaving much room for exploration. In this section, we discuss the antibiotic-resistance mechanisms of *H. pylori* biofilm in conjunction with the latest research advancements.

EPS Matrix Protection

The EPS matrix of *H. pylori* biofilm comprises polysaccharides, extracellular proteins, eDNA, and OMVs. *H. pylori* biofilm is encased in an EPS matrix, which preserves the structural integrity of the biofilm, promotes its adhesion, and facilitates bacterial interactions.[35] Research has shown that the EPS matrix plays a vital role in antibiotic resistance of *H. pylori* biofilm, since the targets of antibiotics are usually located inside bacterial cells and the EPS matrix encases the bacteria and reduces the penetration of antibiotics.[84] Additionally, studies have shown that the EPS matrix typically carries a negative charge, while certain antimicrobial drugs carry a positive charge, so the EPS matrix forms a natural charge barrier and limits the penetration ability of antibiotics and reduces their transport efficiency.[85] Furthermore, due to the barrier protection effect of the EPS matrix, slowly diffusing antibiotics may allow *H. pylori* to undergo adaptive changes, further enhancing antibiotic resistance.[86,87] Therefore, disrupting the EPS matrix of *H. pylori* biofilm could be an extremely important strategy for its effective eradication.[35]

Coccoid Morphology of *H. pylori*

Compared to the spiral *H. pylori* that grows in a planktonic state, the coccoid form is more common in biofilm growth patterns.[16,64] When external conditions are unfavorable for the proliferation of *H. pylori*, such as nutrient scarcity, low oxygen, low acidity, or intervention with antibiotics, *H. pylori* forms biofilm and transforms from a spiral form to coccoid

form.[78] Similarly to other bacteria, the coccoid morphology is in a dormant state, in which the bacteria can maintain slow growth and contribute to the development of antibiotic resistance.[77,88] Studies have shown that when *H. pylori* transitions to a coccoid form, there are significant alterations in the cell wall that are related to the growth of biofilm and the development of antibiotic resistance.[89,90] In *H. pylori* biofilm, the surfaces of coccoid cell walls are modified by peptidoglycan and covered with mucus, which hinders the penetration of antibiotics, leading to antibiotic resistance. Studies have shown that highly expressed undecaprenyl pyrophosphate synthase (*uppS*) genes in biofilm promote the modification of peptidoglycan in the cell wall and ethoxzolamide impedes cell-wall synthesis by competitively inhibiting *uppS*.[91,92] Additionally, certain genes that are highly expressed in coccoid morphology within biofilm play a role in biofilm formation and the emergence of antibiotic resistance, such as *pgdA* (encoding peptidoglycan deacetylase) and *hp0421* (encoding cholesteryl-α-glucoside transferase).[93–96]

Increasing Expression of Efflux Pump Genes

Typically situated on the cellular membrane of *H. pylori*, efflux pumps facilitate the extracellular expulsion of a range of pharmaceutical agents.[97] These efflux pumps can expel antibiotics from the bacteria, reducing antibiotics from within the bacterial cells and thus promoting the development of resistance.[98] The expression of efflux pump genes is observed in both planktonic and biofilm growth modes of *H. pylori*, indicating that efflux pumps are essential throughout the life cycle of *H. pylori*.[17,99] However, some efflux pump–encoding genes are notably overexpressed in *H. pylori* biofilm (eg, *hp0471*, *hp0497*, *hp0605*, *hp0939*, *hp1118*, *hp1165*, *hp1174*, *hp1327*, and *hp1489*), suggesting their pivotal role in the biofilm's antibiotic resistance.[17,64,100] Related studies have shown that the expression of *hp1327* and *hp1489* is significantly upregulated in the *H. pylori* biofilm of the CLR-resistant *H. pylori* strain TK1402.[99] In addition, deletion of the *hp1174* gene leads to *H. pylori* biofilm defects and increased sensitivity to various types of antibiotics.[100] Furthermore, deletion of the *hp0939*, *hp0497*, and *hp0471* genes also results in *H. pylori* biofilm defects.[17]

Changes in Metabolism of *H. pylori* Biofilm

H. pylori is more susceptible to being targeted and inactivated by antibiotics when it is in a state of rapid growth and active metabolism.[101] Research has found that *H. pylori* reduces its metabolic activity within biofilm and simultaneously shifts to a viable but nonculturable state to avoid eradication by antibiotics.[16] A clinical study pointed out that there is variability in biofilm formation among different strains of *H. pylori* and that the ability of *H. pylori* to form strong biofilm is positively correlated with a decrease in its metabolic rate.[102] Furthermore, one study found that in *H. pylori* with biofilm formation, the expression of genes involved in translation and metabolism is reduced and its metabolic activity is significantly lower than that of planktonic bacteria.[16] Additionally, *H. pylori* can also upregulate the expression of certain specific metabolic enzymes to resist some antimicrobial drugs. For example, a mossy stonewort extract containing acetone from South Africa has a bactericidal effect on *H. pylori*. *H. pylori* within biofilm can upregulate the acetone carboxylase gene (*acxA*) to degrade acetone, while the absence of the *acxA* gene leads to defects in biofilm formation.[103] These studies indicate that changes in the metabolic activity of *H. pylori* within biofilm make it less susceptible to eradication and are an important cause of *H. pylori*'s resistance to antimicrobial drugs.

Other Antibiotic-Resistance Mechanisms

Research has shown that some point mutations (positions 2143/2142 in the V structural domain of 23S rRNA) within *H. pylori* biofilm lead to the development of antibiotic resistance.[104] Moreover, antibiotic-resistance genes can develop drug resistance within biofilm through mechanisms such as binding to mobile plasmids, integrating genetic elements, and horizontal gene transfer.[105] Furthermore, eDNA in biofilm can promote microbial adhesion and inhibit the diffusion of antibiotics.[106,107] Additionally, some studies have found that bacteria in biofilm can produce specific enzymes, such as β-lactamases, which hydrolyze antibiotics and render them ineffective.[108] Some of the aforementioned mechanisms in *H. pylori* are not yet fully understood and require further research.

Clinical Drug Treatment of *H. pylori*

Currently, the recommended regimens for *H. pylori* eradication involve standard triple therapy or bismuth quadruple therapy that includes two antibiotics (such as CLR, amoxicillin [AMX], metronidazole, and levofloxacin).[109–111] However, the efficiency of antibiotics in vivo is not as high as that in vitro, because of the acidic environment of the stomach and the inability of antibiotics to effectively target the gastric mucosa.[30] Additionally, the widespread use of antibiotics has led to an increasingly serious issue of antibiotic resistance. A study on antibiotic resistance of *H. pylori* in South Asia showed high resistance rates for metronidazole (69%), CLR (27%), and levofloxacin (34%).[112] A single-center retrospective study in China showed that the proportion of primary and secondary multidrug-resistant isolates of *H. pylori* were 17.8% and 63.2%, respectively.[113] Moreover, the long-term use of antibiotics for *H. pylori* eradication therapy can lead to gut microbiota imbalance, causing discomfort such as diarrhea and bloating, resulting in poor patient compliance.[114] In some patients, this intestinal microbiota disorder may persist even 12 months after treatment.[115–117] Dysbiosis of the intestinal microbiota also poses a serious threat to human immunity, metabolism, and neurological functions.[118] Therefore, there is an urgent need to explore new strategies for treating *H. pylori*, especially those biofilm-forming strains, while achieving high eradication rates and minmizing adverse reactions. In this regard, the development of nanomaterials has opened up new research directions.

Nanomaterial-Based Treatment of Biofilm-Forming *H. pylori*

Nanotechnology represents a cutting-edge field in contemporary science, highlighting the nanoscale size of 1–100 nm.[119,120] Within this dimensional range, materials exhibit physical, chemical, and biological properties that differ significantly from their bulk counterparts. Due to the nanoscale dimensions, large surface area, and high surface-to-volume ratio, nanomaterials exhibit high specificity and distinct characteristics in the biological environment, including in antitumor therapy, drug delivery, and drug-resistant bacteria treatment.[121–123] Owing to the resistance of *H. pylori* biofilm to conventional antibacterial agents, nanomaterial-based treatments for *H. pylori* biofilm have received significant attention. This section outlines the different types of nanomaterials and provides an overview of current studies on the diverse interaction mechanisms of nanomaterials with *H. pylori* biofilm.

Types of Nanomaterial for Treating Biofilm-Forming *H. pylori*

Currently, the most commonly used nanomaterials in treating biofilm-forming *H. pylori* include inorganic NPs, lipid-based NPs, and polymeric NPs.[124] In the following section, we review the antibiofilm effects and mechanisms of different types of NPs.

Inorganic Nanoparticles

Inorganic NPs are primarily derived from metals (metal oxides), silica, and carbon, possess photosensitive, conductive, magnetic, and thermal properties, and can serve as drug carriers and therapeutic agents.[125] In terms of *H. pylori* biofilm eradication, the application of metal-based NPs has been the most extensively studied. The mechanisms by which metal-based NPs eradicate *H. pylori* and its biofilm mainly include the following. 1) Metal ion release: Metal ions diffuse into *H. pylori* and interact with the cell membrane, cell wall, and cellular macromolecules such as proteins and nucleic acids. This leads to the loss of cellular integrity and disruption of the energy transport chain, causing damage to *H. pylori* at both the metabolic and structural levels.[126–128] 2) Reactive oxygen species (ROS) production: Metal-based NPs activate the production of ROS when disrupting bacterial cell walls, nucleic acids, and proteins. High concentrations of ROS can damage the cell membrane through oxidative stress and lipid peroxidation, ultimately leading to bacterial death.[129,130] 3) Stimuli-responsive dynamic effects: The application of infrared light, ultrasound (US), or magnetic fields can drive metal-based NPs to vibrate and generate heat. This not only disrupts mature biofilm but also releases more ROS to damage bacterial structures, achieving effective antibacterial outcomes.[131,132]

Lipid-Based Nanoparticles

Lipid-based NPs are a specific drug-delivery system composed of a lipid matrix surrounded by a surfactant membrane. They have high biocompatibility and can control drug delivery, protect drugs, and target drug delivery, and comprise various types

of liposome, solid-lipid NPs, nanostructured lipid carriers, and nanoemulsions.[133–135] By adjusting the physicochemical properties of lipid-based NPs, their ability to penetrate biofilm structures can be significantly enhanced.[136,137] In addition, cationic lipid-based NPs can interact ionically with negatively charged bacterial cells and fuse with the bacterial cell envelope, making them more effective against negatively charged bacteria.[138] The size of liposomes also affects their ability to penetrate biofilm. Studies have found that liposomes smaller than 500 nm can effectively deliver antimicrobial agents into biofilm.[139] Additionally, by modifying the surface of lipid-based NPs with antibodies or chemical substances, specific targeting to biofilm can be achieved.[140] It is worth noting that lipid-based nanomaterials can enter cells and kill *H. pylori* within both cells and biofilm.[141]

Polymeric Nanoparticles

Polymeric NPs are synthesized by using polymeric materials (such as chitosan [CS], polyamino acid, and polyester) and nanoscale colloidal organic compounds.[142,143] Compared to lipid-based NPs, polymeric NPs have greater loading capacity and mechanical stability.[144] Among them, the most representative is CS. The positively charged CS interacts with the negatively charged bacterial cell surface, leading to cell membrane damage and subsequent leakage of intracellular contents, thereby exerting an antibacterial effect.[145,146] In addition, CS has strong adhesive properties, enabling it to bind with mucins in the biofilm, making it an attractive material for combating bacteria and biofilm.[147] When CS binds to biofilm, it can not only make contact with the surface but also penetrate into the biofilm to kill or damage the bacteria within.[148] Arif et al synthesized a rhamnolipid (RHL)–CS hybrid NP that effectively degraded *H. pylori* biofilm, achieving an eradication rate of up to 96% for *H. pylori* within the biofilm.[149]

Mechanisms of Nanomaterials in the Treatment of Biofilm-Forming *H. pylori*

Understanding how nanomaterials engage in *H. pylori* biofilm formation is of great significance for the development of nanomaterials. Based on the aforementioned mechanisms of *H. pylori* biofilm formation, nanomaterials can play a disruptive role at various stages, such as inhibiting biofilm formation, improving biofilm penetration, destroying mature biofilm, and serving as drug-delivery systems to enhance drug efficacy. The mechanisms by which nanomaterials treat *H. pylori* biofilm are further categorized and summarized in the following subsections.

Inhibition of *H. pylori* Biofilm Formation

Nanomaterials can impede the formation of *H. pylori* biofilm by inhibiting adhesion of *H. pylori*. In our previous study, we synthesized nanoclusters (NCs) based on $Zn_{0.3}Fe_{2.7}O_4$ NPs using poly(ethylene glycol)-*b*-poly(ε-caprolactone) as the nanocarrier. The biocompatibility of the NCs was higher than that of the NPs. The photothermal effect of NCs enhanced the susceptibility of *H. pylori* to antibiotics, potentially by impeding biofilm formation and reducing bacterial adhesion (Figure 3A).[150] Additionally, Shu et al developed a mucus-penetrating therapeutic platform (Cu-MOF@NF) based on copper-containing metal–organic frameworks (Cu-MOFs) integrated with carbon dots and the polysaccharide fucoidan (FU). They found that Cu-MOF@NF is capable of penetrating the mucus barrier, preventing *H. pylori*'s adherence to gastric epithelial cells to inhibit biofilm formation and releasing Cu^{2+} to degrade polysaccharides within the biofilm. The platform not only had high antibacterial efficiency but also improved the inflammatory microenvironment compared to antibiotics without disrupting the balance of the gut microbiota (Figure 3B).[151] In addition, Camargo et al synthesized silver-containing compounds and loaded them into polymer (poly[ε-caprolactone]) NPs. This nanomaterial not only inhibited the formation of *H. pylori* biofilm but also had a destructive effect on mature biofilm.[152]

In a study conducted by Zhang et al, the authors utilized *H. pylori* OMVs as a source of membrane, which contains adhesion and pathogenic factors similar to those of intact *H. pylori*. A polymer nanocore was then prepared from poly (lactic-*co*-glycolic acid) (PLGA) and bacterium-mimicking NPs (OM-coated NPs) were synthesized by fusing OMVs onto the PLGA nanocore using US. OM-coated NPs bound to gastric epithelial cells to compete for binding sites with *H. pylori*, thereby inhibiting the pathogenic adhesion of *H. pylori*.[153] Arif et al conducted a reductive reaction with CS and mannose, followed by an ionic gelation reaction to prepare mannose-functionalized CS NPs. These NPs were capable of impeding the adhesion of *H. pylori* and reducing its biofilm-forming ability by disrupting bacterial pili and flagella.[154]

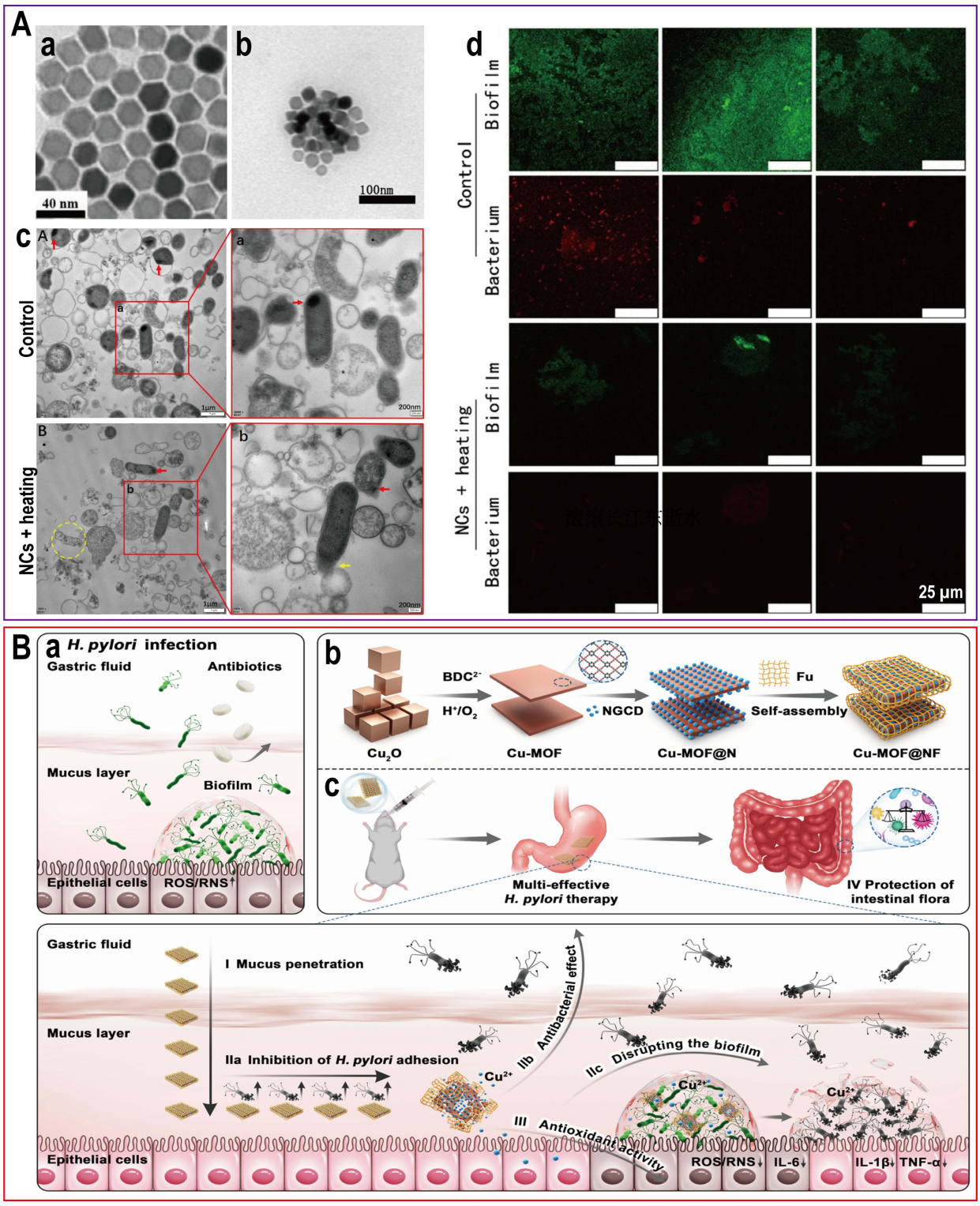

Figure 3 Nanomaterial-based treatment of *H. pylori* biofilm through inhibition of its formation. (**A**) The suppressive impact of nanoclusters (NCs) on the formation of *H. pylori* biofilm: (a) SEM image of $Zn_{0.3}Fe_{2.7}O_4$ NPs; (b) TEM image of NCs; (c) photothermal effects of NCs on *H. pylori* morphology; (d) photothermal effects of NCs inhibiting the formation of *H. pylori* biofilm. Reproduced from Meng F, Tao H, Mi Y, et al. Nanocluster-mediated photothermia improves eradication efficiency and antibiotic sensitivity of *Helicobacter pylori*. Cancer Nanotechnol. 2022;13(1):13. Creative Commons Attribution 4.0.[150] (**B**) Schematic illustration of the synthesis of Cu-MOF@NF and mechanism of treating *H. pylori* biofilm: (a) formation of *H. pylori* biofilm in the stomach; (b) synthesis process of Cu-MOF@NF; (c) mechanism of treating *H. pylori* biofilm and improving the inflammatory microenvironment. Reproduced from Shu C, Zhang W, Zhang Y, et al. Copper-bearing metal-organic framework with mucus-penetrating function for the multi-effective clearance of mucosal colonized *Helicobacter pylori*. Research. 2024;7:0358. Copyright © 2024 Chunxi Shu et al. Exclusive licensee Science and Technology Review Publishing House. No claim to original U.S. Government Works. Distributed under a Creative Commons Attribution License 4.0 (CC BY 4.0).[151]

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Enhancement of *H. pylori* Biofilm Permeability

Enhancing bacterial biofilm permeability relies mainly on the size, surface modification, surface potential, and other characteristics of nanomaterials.[123,155,156] Zou et al utilized an assembly of metformin linoleic acid (ML) and linoleic acid (LA) to synthesize FU-coated NPs with an outer layer coated with the urease inhibitor ebselen (EB) (FU–ML–LA–EB NPs; Figure 4A). The negatively charged FU–ML–LA–EB NPs readily penetrated the gastric mucus barrier to reach the site of infection, and EPS disrupted the biofilm structure. The FU–ML–LA–EB NPs also alleviated oxidative stress, thereby reducing gastric mucosal injury and interrupting the pathway of carcinogenesis.[36] Additionally, Li et al encapsulated docosahexaenoic acid (DHA) in nanostructured lipid carriers (NLCs) to form DHA-loaded NLC (DHA NLCs) (Figure 4B). DHA was proven to be effective against *H. pylori*, both in vitro and in vivo. Within 24 h of interaction with *H. pylori* biofilm, the DHA NLCs were able to penetrate the biofilm, resulting in a reduction in total biofilm biomass. This mechanism may involve the particle size (350 nm) of DHA NLCs enhancing *H. pylori* biofilm permeability.[157] Chen et al synthesized RHL/CLR–cholesterol–calcitriol, the product having a hydrophilic surface, a negative charge, and the ability to rapidly penetrate the gastric mucus layer to reach the site of *H. pylori* infection. RHL enhanced the permeability of biofilm, exposing the internal *H. pylori* while releasing CLR to kill *H. pylori* within the biofilm. Cholesterol stabilized the formulated structure and repaired cell membranes, while calcitriol restored lysosomal acidification.[158]

Disruption of Mature *H. pylori* Biofilm

Nanomaterials can directly disrupt mature *H. pylori* biofilm through mechanisms such as sonodynamic therapy, phototherapy, and targeting of the biofilm matrix. In recent years, sonodynamic therapy based on nanomaterials for treating *H. pylori* biofilm has been extensively researched.[159–161] Yin et al reported a targeted nanoplatform based on sonodynamic therapy (Figure 5A). Based on the covalent bonding of CS and fructose, a nanoshell (FCS) was formed and indocyanine

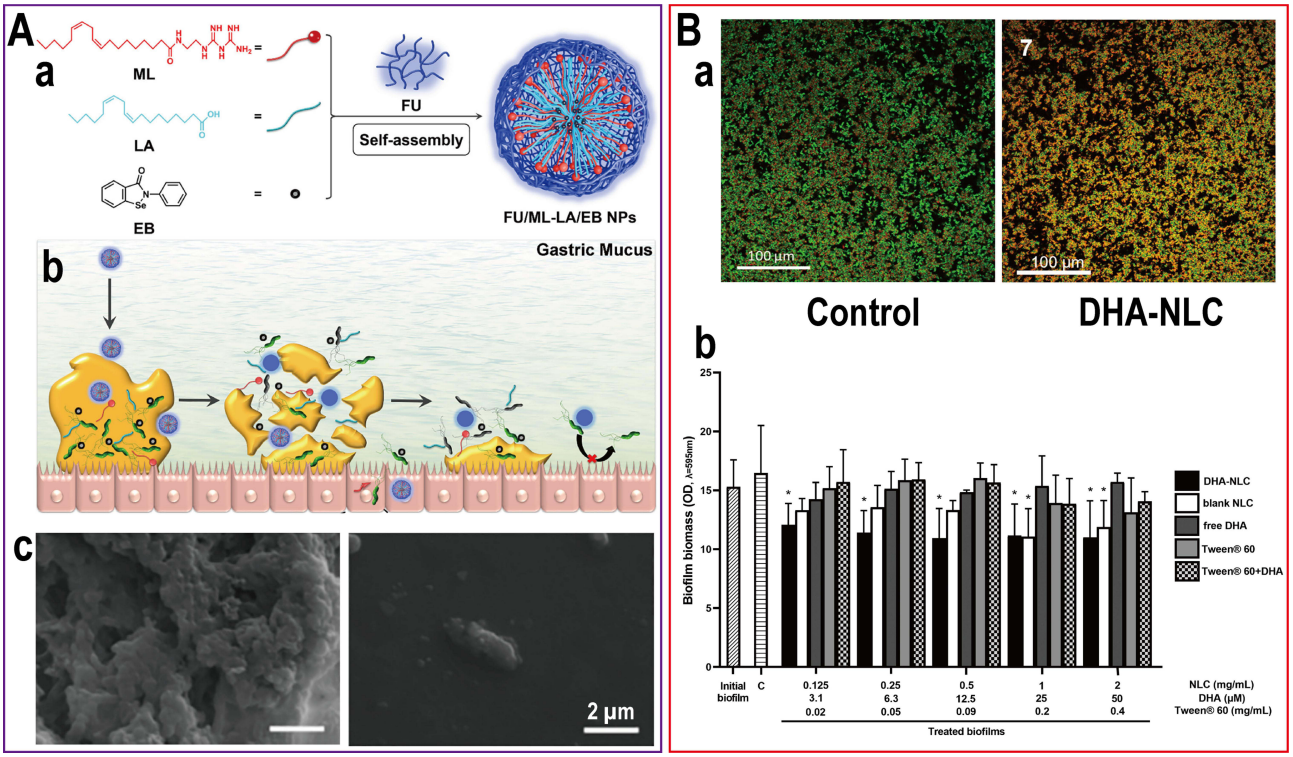
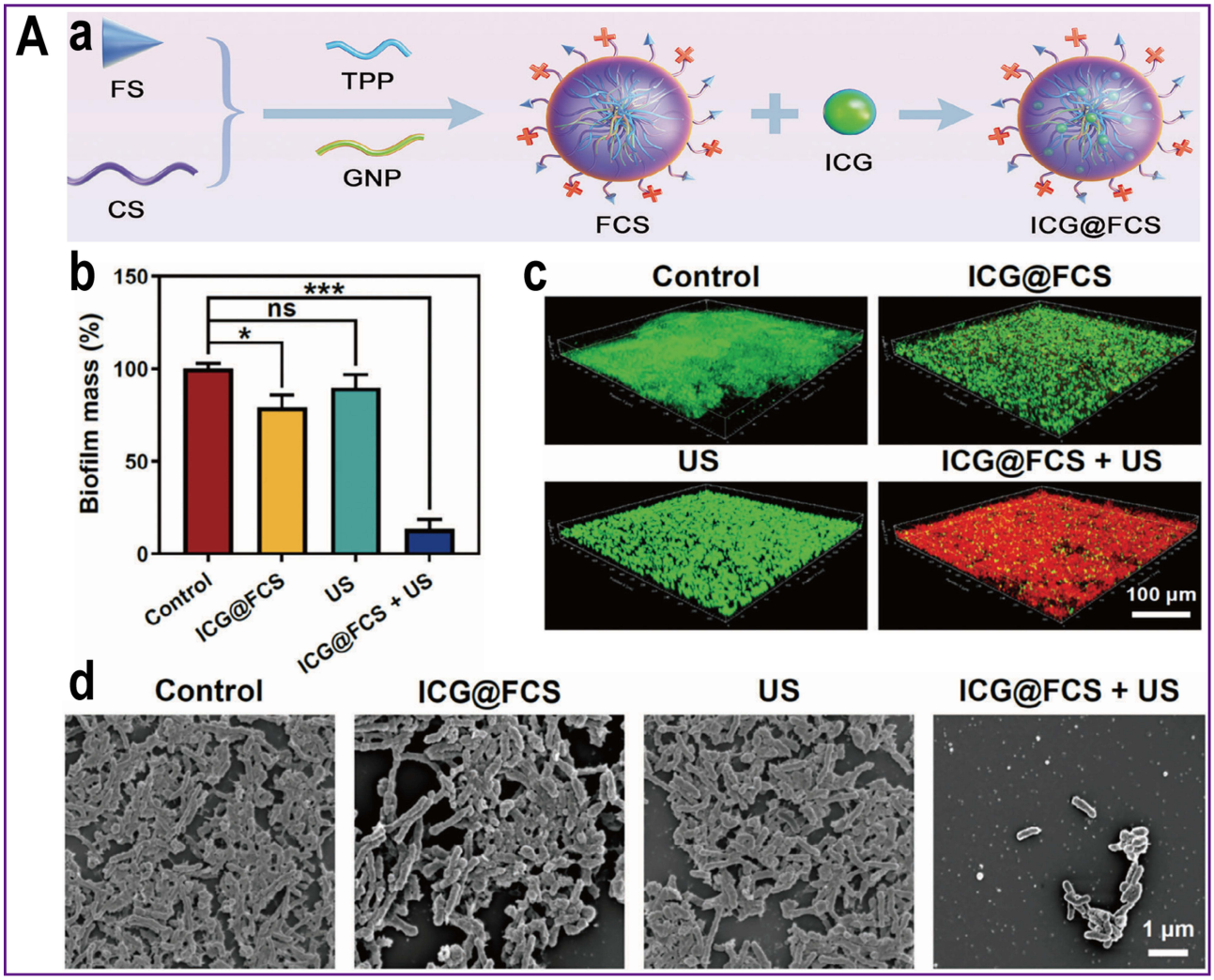


Figure 4 Nanomaterial-based treatment of *H. pylori* biofilm through enhancement of biofilm permeability. (**A**) Synthesis of FU–ML–LA–EB NPs and enhancement of *H. pylori* biofilm permeability: (a) synthesis of FU–ML–LA–EB NPs; (b) schematic diagram of FU–ML–LA–EB NP-enhanced *H. pylori* biofilm permeability; (c) SEM of changes in *H. pylori* biofilm after FU–ML–LA–EB NP treatment. Reproduced from Zou Y, Chen X, Sun Y, et al. Antibiotics-free nanoparticles eradicate *Helicobacter pylori* biofilms and intracellular bacteria. J Control Release. 2022;348:370–385. Copyright (2022), with permission from Elsevier.[36] (**B**) Effects of DHA-NLC on *H. pylori* biofilm by (a) CLSM and (b) biofilm biomass. **p*<0.05 compared with the control group. Reproduced from Pinho AS, Seabra CL, Nunes C, et al. *Helicobacter pylori* biofilms are disrupted by nanostructured lipid carriers: a path to eradication? J Control Release. 2022;348:489–498 Copyright (2022), with permission from Elsevie.[157]

green (ICG) encapsulated within it to synthesize the final ICG@FCS platform. ICG@FCS penetrated the gastric mucosa and locked onto *H. pylori* through a molecular targeting mechanism via fructose and physical targeting mechanism via US. Upon US activation, it generated singlet oxygen, effectively attacking planktonic *H. pylori* and destroying *H. pylori* biofilm. This ICG@FCS nanoplatform had minimal effect on the intestinal microbiota and contributed to the repair of the gastric mucosa.[159] In addition, Fan et al constructed $PtCu_3$–PDA@AIPH@fucoidan (PPAF) NPs capable of generating alkyl radicals (R·). This nanomaterial was composed of $PtCu_3$, a sonosensitizer modified by dopamine, AIPH, an alkyl radical generator, and fucoidan, which targets *H. pylori*. The surface-modified fucoidan endowed the PPAF with good hydrophilicity and a negative charge, which facilitated the penetration of NPs through the gastric mucus barrier. It targeted fucoidan-binding proteins on the surface of *H. pylori*, thereby enhancing antibacterial efficiency. Under US, AIPH can generate R, thus exerting antibacterial effects in the hypoxic environment where *H. pylori* colonizes. While generating R, AIPH can also produce nitrogen gas, which disrupts mature biofilm and promotes the binding of NPs to *H. pylori*. Compared to antibiotic therapy, PPAF based on sonodynamic therapy helped regulate the inflammatory environment caused by *H. pylori* infection without causing dysbiosis of the gut microbiota.[160]

Additionally, RHL produced by *Burkholderia* or *Pseudomonas* can disrupt *H. pylori* biofilm through the sequestration of metal ions in the EPS matrix and impede signal transduction, which is essential for biofilm formation.[35,163–165] Li et al described a multifunctional antibiotic-free copper–organic framework (HKUST-1) platform (Figure 5B), which was



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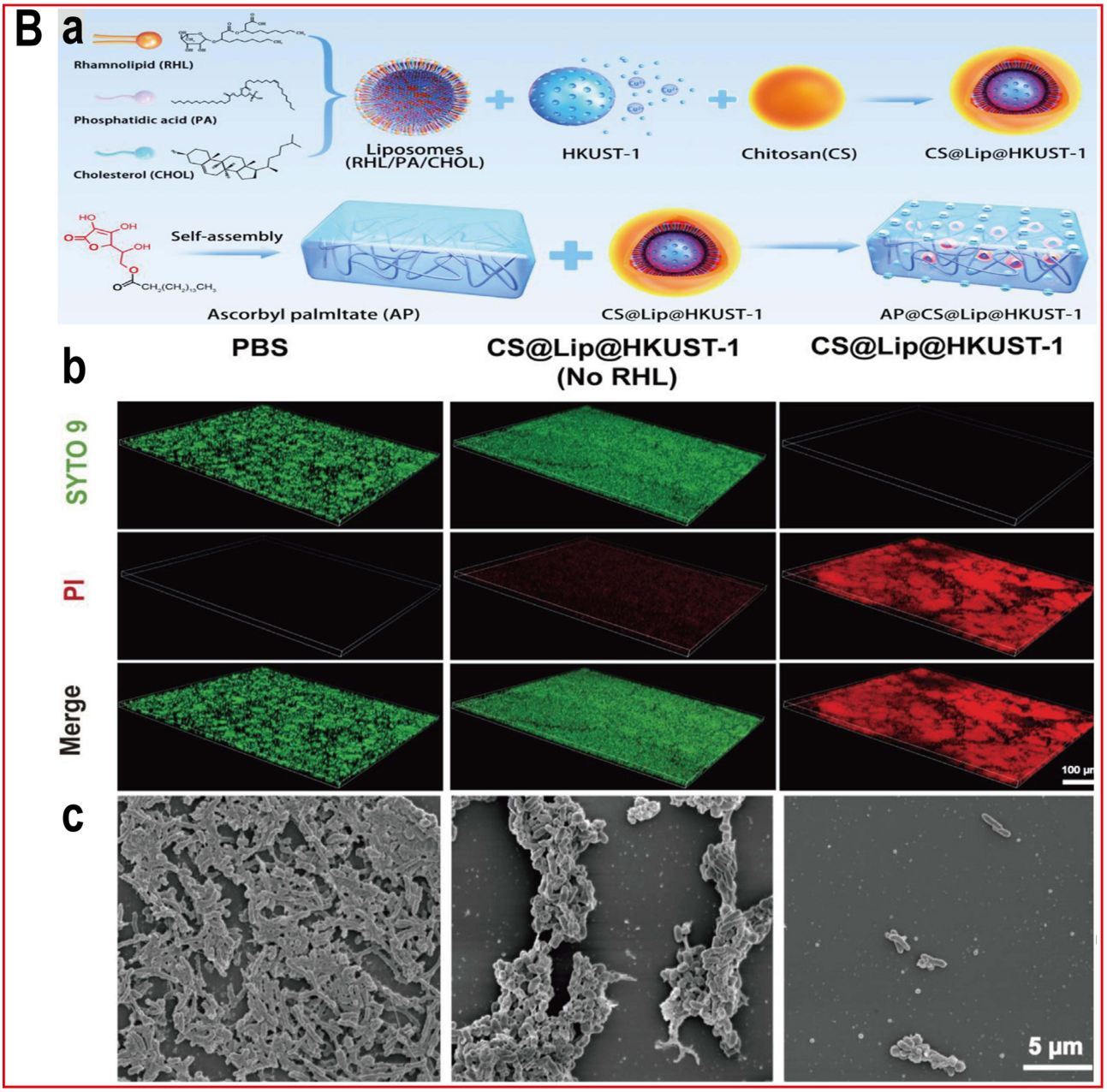


Figure 5 Nanomaterial-based treatment of *H. pylori* biofilm through disruption of mature biofilm. (**A**) Synthesis of ICG@FCS and disruption of mature *H. pylori* mature biofilm: (a) synthesis of ICG@FCS —detecting the impact of sonodynamic therapy of ICG@FCS on *H. pylori* biofilm through (b) biofilm mass, (c) CLSM, and (d) SEM. **p*<0.05, ****p*<0.001. ns, not significant compared with the control group. Reproduced from Yin X, Lai Y, Zhang X, et al. Targeted sonodynamic therapy platform for holistic integrative *Helicobacter pylori* therapy. Adv Sci. 2024:e2408583. © 2024 The Author(s). Advanced Science published by Wiley-VCH GmbH CCBY license.[159] (**B**) Synthesis of AP@CS@Lip@HKUST-1 and disruption of mature *H. pylori* biofilm: (a) synthesis of AP@CS@Lip@HKUST-1 — detecting the impact of CS@Lip@HKUST-1 on *H. pylori* mature biofilm through (b) CLSM and (c) SEM. Reproduced from Lai Y, Zhang T, Yin X, et al. An antibiotic-free platform for eliminating persistent *Helicobacter pylori* infection without disrupting gut microbiota. Acta Pharm Sin B. 2024;14(7):3184–3204. © 2024 The Authors. CC BY-NC-ND license.[162]

encapsulated in a lipid layer composed of RHL, phosphatidic acid (PA), and cholesterol (CHOL), further encapsulated with CS, and loaded into an ascorbyl palmitate (AP) hydrogel, ultimately forming AP@CS@Lip@HKUST-1. This platform targeted inflammatory sites through electrostatic attraction, and the CS-encapsulated NPs were released by hydrolysis of matrix metalloproteinases, disrupting *H. pylori* urease activity and dispersing the biofilm.[162]

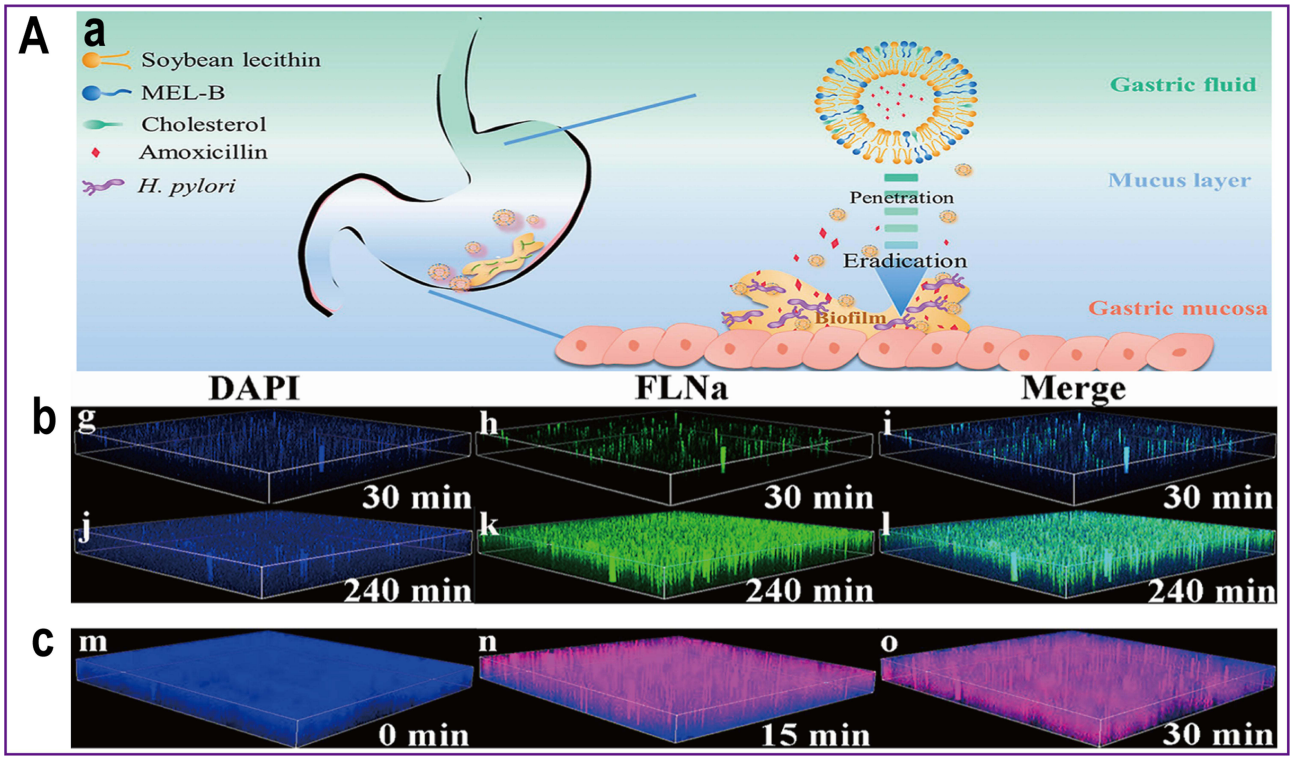
Phototherapy, including photothermal therapy and photodynamic therapy, is widely applied in the treatment of *H. pylori* biofilm.[37] Qiao et al designed a phototherapy nanomedicine (RLs@T780TG) targeting multidrug-resistant *H.*

pylori infection composed of a near-infrared photosensitizer T780T-Gu and an anionic component — RHL. Under near-infrared (808 nm) irradiation, RLs@T780TG decomposed and T780T-Gu disrupted the polysaccharides and proteins in the EPS through photothermal therapy and ROS generation (photodynamic therapy). Compared to antibiotic treatment, gastric mucosal lesions were significantly alleviated, and their impact on the gut bacterial balance was minimal.[166]

Drug-Delivery Systems

Drug-delivery systems based on nanomaterials can encapsulate antibiotics or natural antimicrobial agents, thereby protecting them from inactivation induced by enzymatic reactions.[167] Additionally, drug-delivery systems can exert synergistic effects to enhance antimicrobial efficiency and reduce drug toxicity through precise drug release.[168] In this context, many studies have focused on drug delivery systems using nanomaterials to carry CLR, AMX, berberine, curcumin, and other substances to enhance the treatment of *H. pylori* biofilm.[38,44,169,170] Wu et al developed a novel nanoliposome (LipoSC-MELB) using mannosylerythritol lipid B, soybean lecithin, and cholesterol as raw materials, which are stable in gastric acid (Figure 6A). They then incorporated AMX into the LipoSC-MELB to form LipoSC-MELB–AMX, with an average diameter of approximately 100 nm. LipoSC-MELB–AMX was capable of carrying AMX through the barriers of gastric mucus and *H. pylori* biofilm, and had greater anti–*H. pylori* biofilm ability than free AMX.[171]

Additionally, Li et al prepared LPNs with CS NPs as the core and RHL as the shell. They then modified the surface of LPNs with DSPE–PEG^{2000} to enhance hydrophilicity and encapsulate CLR within the LPNs (Figure 6B). LPNs were more efficient in eradicating *H. pylori* biofilm than free CLR. In this system, RHL inhibited bacterial adhesion and disrupted EPS in the biofilm, whereas CLR and CS NPs killed *H. pylori* within the biofilm. Additionally, PEGylated LPNs penetrated mucus rapidly and effectively cleared *H. pylori* biofilm beneath the mucous layer.[35] Huang et al synthesized a multifunctional nanomedicine composed of an RHL-assisted black phosphorus nanocomposite (RHL@BP/ISL) for the delivery of isolinderalactone (ISL). ISL has demonstrated



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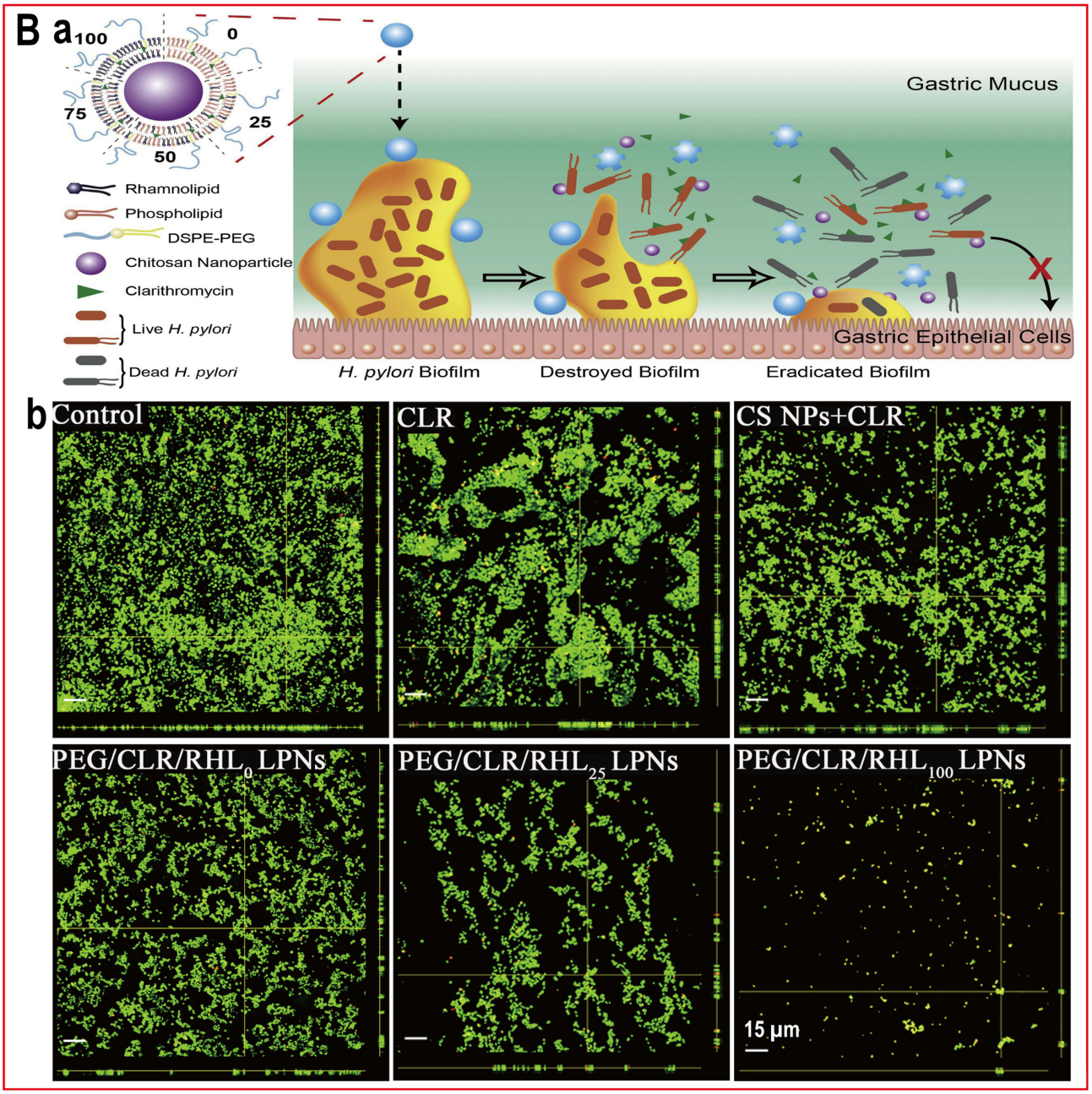

Figure 6 Nanomaterial-based treatment of *H. pylori* biofilm through drug-delivery system. (**A**) Synthesis of LipoSC-MELB–AMX and mechanism of anti–*H. pylori* biofilm: (a) schematic diagram of the composition of LipoSC-MELB–AMX and its movement across the gastric mucus barrier and *H. pylori* biofilm; (b) fluorescein sodium (green)-labeled LipoSC-MELB–AMX'penetration in the gastric mucosa of mice by CLSM (30 minutes and 240 minutes after gavage); (c) *H. pylori* biofilm penetration of LipoSC-MELB encapsulated with Neil red by CLSM. Reproduced from Wu Y, Geng J, Cheng X, et al. Cosmetic-derived mannosylerythritol lipid-B-phospholipid nanoliposome: an acid-stabilized carrier for efficient gastromucosal delivery of amoxicillin for in vivo treatment of *Helicobacter pylori*. ACS Omega. 2022;7(33):29086–29099. Copyright © 2022 The Authors. Published by American Chemical Society. CC-BY-NC-ND 4.0.[171] (**B**) Synthesis of LPNs and mechanism of anti–*H. pylori* biofilm: (a) schematic diagram of the structure of LPNs and the process of treating *H. pylori* biofilm (numbers surrounding LPNs) indicate the amount of RHL (%); (b) effects of LPNs on the viability and structure of *H. pylori* biofilm. Reproduced from Li P, Chen X, Shen Y, et al. Mucus penetration enhanced lipid polymer nanoparticles improve the eradication rate of *Helicobacter pylori* biofilm. J Control Release. 2019;300:52–63. Copyright 2019, with permission from Elsevier.[35]

good antibacterial activity against *H. pylori* in vitro. Black phosphorus is acid-sensitive, while RHL is unstable under light and heat. Under near-infrared light irradiation, the controllable and effective release of ISL is triggered for photothermal therapy, which reduces the adhesion of *H. pylori* and inhibits biofilm formation.[172]

Limitations of Nanomaterials in the Treatment of Biofilm-Forming *H. pylori*

Despite the advantages of nanomaterials in treating *H. pylori* biofilm, there are still limitations. First, the stability of metal-based NPs is relatively insufficient, especially in environments such as gastric acid. Moreover, while metal-based NPs can kill *H. pylori*, they may also exhibit biological toxicity. For example, excessive copper ions can lead to copper poisoning.[173] Additionally, some metal-based NPs are costly. Gold and silver, for instance, have good antibacterial effects against *H. pylori*, but their large-scale production may not be cost-effective.[174] Lipid-based NPs have certain shortcomings. Their physicochemical properties are unstable and are easily affected by pH and temperature.[175,176] Furthermore, due to the sensitivity of lipids to high temperatures, special sterilization techniques are required, and completely eliminating pathogens is relatively difficult.[177] Polymeric NPs also have certain limitations. In addition to the potential for cytotoxicity, positively charged polymer materials actively interact with cellular proteins, which reduces their antibacterial and antibiofilm efficiency in vivo.[178]

Conclusion and Outlook

Antibiotic resistance and high eradication-failure rates are severe challenges currently faced by *H. pylori* treatment strategies. *H. pylori* can form biofilm not only in vitro but also in vivo, and the formation of biofilm is closely related to its antibiotic resistance. Mechanisms such as the barrier effect of biofilm, the involvement of efflux pumps, and changes in bacterial metabolic activity within biofilm lead to drug resistance and limit the clinical application of antibiotic-based eradication regimens. Moreover, the use of antibiotics not only exacerbates drug resistance but also causes a variety of adverse reactions, leading to poor patient compliance. Therefore, exploring antibiotic-independent strategies for treating *H. pylori* biofilm is an important direction for addressing the challenges of biofilm-forming *H. pylori* eradication.

Nanomaterials, with their unique physicochemical properties and highly efficient antibiofilm mechanisms, have emerged as a promising new strategy. Nanomaterials can eradicate biofilm-forming *H. pylori* through various mechanisms, including preventing biofilm formation, improving biofilm permeability, disrupting mature biofilm, and serving as a drug-delivery system that synergizes with antimicrobials. Based on their type, nanomaterials offer certain advantages to eradicating biofilm-forming *H. pylori*: 1) inorganic NPs possess photosensitivity, magnetism, and thermal properties, which enable them to directly disrupt *H. pylori* biofilm through physical mechanisms; 2) lipid-based nanomaterials show strengths in *H. pylori* biofilm penetration, high biocompatibility, and drug-loading capacity; and 3) polymeric NPs offer superior functionalization, stability, and pH responsiveness. However, nanomaterials also have certain limitations. The cost of metal NPs needs to be reduced, the stability of lipid NPs needs to be improved, and the biocompatibility of polymer NPs needs to be further enhanced. Therefore, further research is needed to integrate the advantages of various types of nanomaterial for efficient *H. pylori* biofilm treatment, such as targeting biofilm-specific metabolites or working synergistically with antibiotics.

Although existing studies have confirmed that nanomaterials have significant effects and potential in eradicating *H. pylori* biofilm, their application in clinical practice still faces numerous obstacles. First, the design of nanomaterials must take into account the complex physiological environment of the human body to ensure that they are safe, atoxic, and biodegradable. In addition, the processes of drug administration, metabolism, and excretion of nanomaterials need to be optimized and validated to maximize their antibiofilm efficacy and biosafety. At the same time, simplifying the synthesis steps and reducing costs to facilitate large-scale production are also crucial. In summary, with continuous optimization, nanomaterials hold promise as part of a new generation of strategies for treating biofilm-forming *H. pylori* and are expected to ultimately achieve the eradication of *H. pylori* in the near future.

Data Sharing

No data were used for the research described in this study.

Acknowledgments

We thank all authors for their help and contributions to this study. The authors would like to thank FigDraw (www.figdraw.com) for picture source assistance during the preparation of Figure 2.

Funding

There is no funding to report.

Disclosure

The authors declare that they have no conflicts of interest.

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