


# 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.<sup>1–4</sup> *H. pylori* is a significant contributor to a broad range of gastrointestinal diseases, including chronic gastritis, peptic ulcer disease, autoimmune diseases, and gastric adenocarcinoma.<sup>5,6</sup> In 1994, *H. pylori* was classified as a class I carcinogen by the World Health Organization.<sup>7</sup> 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.<sup>8</sup> 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.<sup>9–11</sup> 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.<sup>12,13</sup> Among the various antibiotic-resistance mechanisms of *H. pylori*, the formation of bacterial biofilm is an important factor.<sup>14</sup> 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.<sup>15,16</sup> Moreover, efflux pump genes that form biofilm are highly expressed in *H. pylori*, promoting the development of antibiotic resistance.<sup>17</sup> Furthermore,

the morphology of *H. pylori* within biofilm changes from spiral to coccoid form, leading to increased tolerance to antibiotics.<sup>18</sup> 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.<sup>19–21</sup> Studies have shown that the antimicrobial peptides DJK-5 and IDR-1018 can inhibit the growth and maturation of *H. pylori* biofilm.<sup>22</sup> 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.<sup>23,24</sup> 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.<sup>25</sup> 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.<sup>26–28</sup> Nanomaterials consist of particles with dimensions  $\leq 100$  nm.<sup>29</sup> 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).<sup>30</sup> 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.<sup>31–33</sup> 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.<sup>34</sup> 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.<sup>35</sup> Polymer NPs can enhance the permeability of *H. pylori* biofilm by adjusting their surface properties.<sup>36</sup> Inorganic NP-based photothermal therapy/photodynamic therapy can directly destroy mature *H. pylori* biofilm by interacting with the biofilm through oxidative stress and hyperthermia.<sup>37</sup> 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.<sup>38,39</sup>

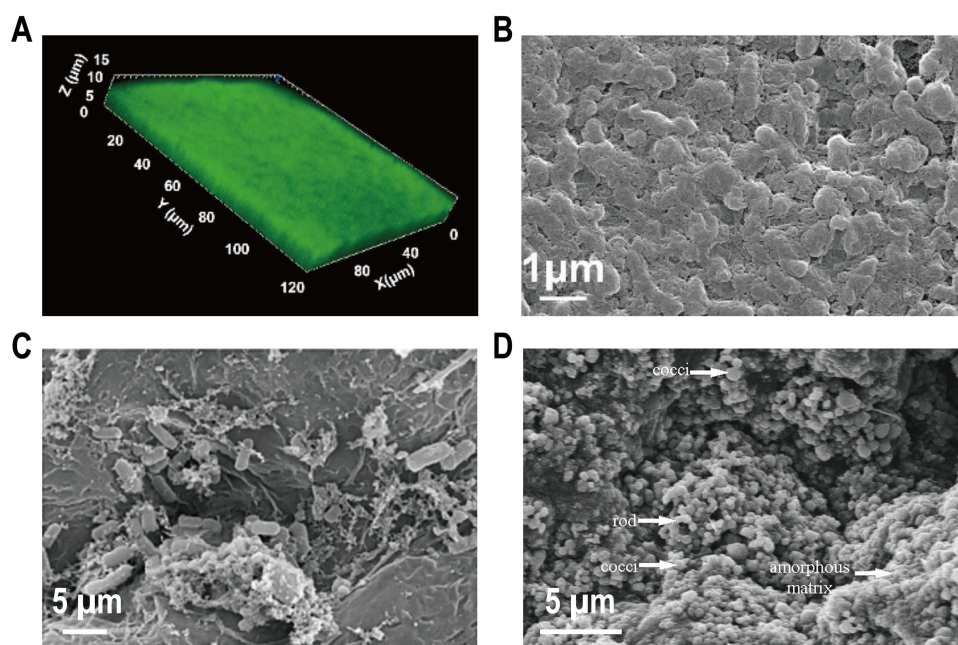
In this review, we first provide a brief introduction to the biological traits of *H. pylori* biofilm and highlight its fundamental mechanisms of formation. We review current studies on the mechanisms of *H. pylori* biofilm-enhancing antibiotic resistance. Furthermore, we discuss advancements in research 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.<sup>40–42</sup> Researchers have indicated that *H. pylori* can form biofilm in both vitro (Figure 1A and B) and vivo (Figure 1C and D) settings.<sup>43–45</sup> 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.<sup>46</sup> 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).<sup>22,47</sup>

Polysaccharides, including glucose, *N*-acetylglucosamine, fucose, and galactose, are ideal molecules for promoting bacterial adhesion and forming the three-dimensional scaffold of biofilm.<sup>29,48–50</sup> Proteins contribute to the stabilization of the matrix network formed by polysaccharides and enhance the binding between bacteria.<sup>51</sup> 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.<sup>43</sup> (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 Elsevier.<sup>43</sup> (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 Elsevier.<sup>44</sup> (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 Elsevier.<sup>45</sup>

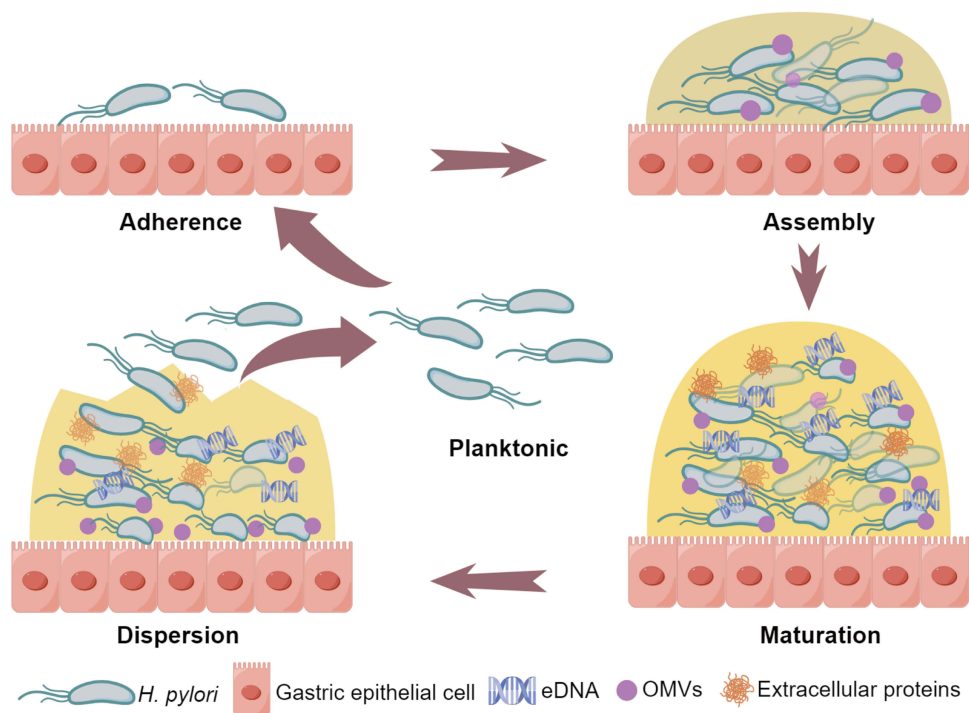
proteins are essential for the EPS within *H. pylori* biofilm and that protease treatment of the biofilm significantly leads to its dispersion.<sup>16,22</sup> The presence of eDNA within the biofilm matrix of various bacterial species has been identified, and it is believed to contribute to the biofilm's structure, facilitate genetic exchange, and influence bacterial diversity.<sup>52,53</sup> Nevertheless, the function of eDNA within *H. pylori* biofilm remains to be comprehensively elucidated,<sup>54</sup> but research suggests it may be related to the aggregation of OMVs.<sup>55</sup> OMVs are double-layered structures with diameters ranging from 20 to 500 nm, and contain proteins, phospholipids, DNA, and lipopolysaccharides.<sup>56</sup> OMVs have been demonstrated to be a part of the biofilm matrix in some Gram-negative bacteria, including *H. pylori*.<sup>57,58</sup> 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.<sup>58,59</sup> 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).<sup>60</sup> 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.<sup>61,62</sup> Research has found that *H. pylori* can adhere not only to gastric epithelial cells but also to nonbiological surfaces.<sup>63–65</sup> 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.<sup>66</sup> 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.<sup>67,68</sup> *H. pylori* can also



**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.<sup>63,69</sup> 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.<sup>62,65,70</sup>

### 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.<sup>64,71,72</sup> 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.<sup>73</sup> 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.<sup>22</sup> 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.<sup>61</sup> Comparative genomics studies further indicate that the assembly speed of *H. pylori* biofilm is similar among different strains.<sup>62</sup> 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.<sup>22</sup>

### Maturation

Mature *H. pylori* biofilm undergoes notable morphological changes, coincident with a marked increase in EPS synthesis.<sup>74</sup> The maturation process of *H. pylori* biofilm is intricately linked to the formation of EPS, which is vital for biofilm development.<sup>16</sup> 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.<sup>16,61,65,75,76</sup> 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.<sup>16,64</sup> Research has indicated that *H. pylori* mutants deficient in flagella production ( $\Delta$ *fliM*,  $\Delta$ *fliA*) have diminished capacity to develop biofilm, which is partly due to the decrease in flagellar filaments within the biofilm structure.<sup>16</sup> *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.<sup>64</sup> In mature *H. pylori* biofilm grown on nonbiological surfaces, a majority of *H. pylori* is in coccoid form, with a minority being rod-shaped.<sup>64</sup> The coccoid morphology of *H. pylori*, once considered a stress-induced response, has mechanisms that remain largely unexplored and not completely understood.<sup>77</sup> 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.<sup>78</sup> 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.<sup>74</sup>

## 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.<sup>16,64,79</sup> 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.<sup>72</sup> 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.<sup>80</sup> Additionally, research has demonstrated that AI-2 promotes the dispersion of *H. pylori* biofilm and an absence of the *luxS* gene ( $\Delta$ *luxS*) leads to a significant increase in biofilm formation compared to the wild-type *H. pylori* strain.<sup>72</sup> 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  $\Delta$ *cheA* exhibits a biofilm phenotype similar to that of the  $\Delta$ *luxS* mutant.<sup>72</sup> 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.<sup>81</sup> Compared to planktonic *H. pylori*, its biofilm state increases adaptive resistance to antibiotics 10–1000-fold.<sup>82,83</sup> 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.<sup>35</sup> 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.<sup>84</sup> 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.<sup>85</sup> 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.<sup>86,87</sup> Therefore, disrupting the EPS matrix of *H. pylori* biofilm could be an extremely important strategy for its effective eradication.<sup>35</sup>

## 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.<sup>16,64</sup> 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.<sup>78</sup> 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.<sup>77,88</sup> 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.<sup>89,90</sup> 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*.<sup>91,92</sup> 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- $\alpha$ -glucoside transferase).<sup>93–96</sup>

## 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.<sup>97</sup> These efflux pumps can expel antibiotics from the bacteria, reducing antibiotics from within the bacterial cells and thus promoting the development of resistance.<sup>98</sup> 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*.<sup>17,99</sup> 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.<sup>17,64,100</sup> 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.<sup>99</sup> In addition, deletion of the *hp1174* gene leads to *H. pylori* biofilm defects and increased sensitivity to various types of antibiotics.<sup>100</sup> Furthermore, deletion of the *hp0939*, *hp0497*, and *hp0471* genes also results in *H. pylori* biofilm defects.<sup>17</sup>

## 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.<sup>101</sup> 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.<sup>16</sup> 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.<sup>102</sup> 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.<sup>16</sup> 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 (*acx4*) to degrade acetone, while the absence of the *acx4* gene leads to defects in biofilm formation.<sup>103</sup> 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.<sup>104</sup> 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.<sup>105</sup> Furthermore, eDNA in biofilm can promote microbial adhesion and inhibit the diffusion of antibiotics.<sup>106,107</sup> Additionally, some studies have found that bacteria in biofilm can produce specific enzymes, such as  $\beta$ -lactamases, which hydrolyze antibiotics and render them ineffective.<sup>108</sup> 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).<sup>109–111</sup> 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.<sup>30</sup> 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%).<sup>112</sup> 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.<sup>113</sup> 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.<sup>114</sup> In some patients, this intestinal microbiota disorder may persist even 12 months after treatment.<sup>115–117</sup> Dysbiosis of the intestinal microbiota also poses a serious threat to human immunity, metabolism, and neurological functions.<sup>118</sup> 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 minimizing 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.<sup>119,120</sup> 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.<sup>121–123</sup> 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.<sup>124</sup> 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.<sup>125</sup> 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.<sup>126–128</sup> 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.<sup>129,130</sup> 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.<sup>131,132</sup>

#### 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.<sup>133–135</sup> By adjusting the physicochemical properties of lipid-based NPs, their ability to penetrate biofilm structures can be significantly enhanced.<sup>136,137</sup> 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.<sup>138</sup> 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.<sup>139</sup> Additionally, by modifying the surface of lipid-based NPs with antibodies or chemical substances, specific targeting to biofilm can be achieved.<sup>140</sup> It is worth noting that lipid-based nanomaterials can enter cells and kill *H. pylori* within both cells and biofilm.<sup>141</sup>

### Polymeric Nanoparticles

Polymeric NPs are synthesized by using polymeric materials (such as chitosan [CS], polyamino acid, and polyester) and nanoscale colloidal organic compounds.<sup>142,143</sup> Compared to lipid-based NPs, polymeric NPs have greater loading capacity and mechanical stability.<sup>144</sup> 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.<sup>145,146</sup> 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.<sup>147</sup> 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.<sup>148</sup> 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.<sup>149</sup>

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

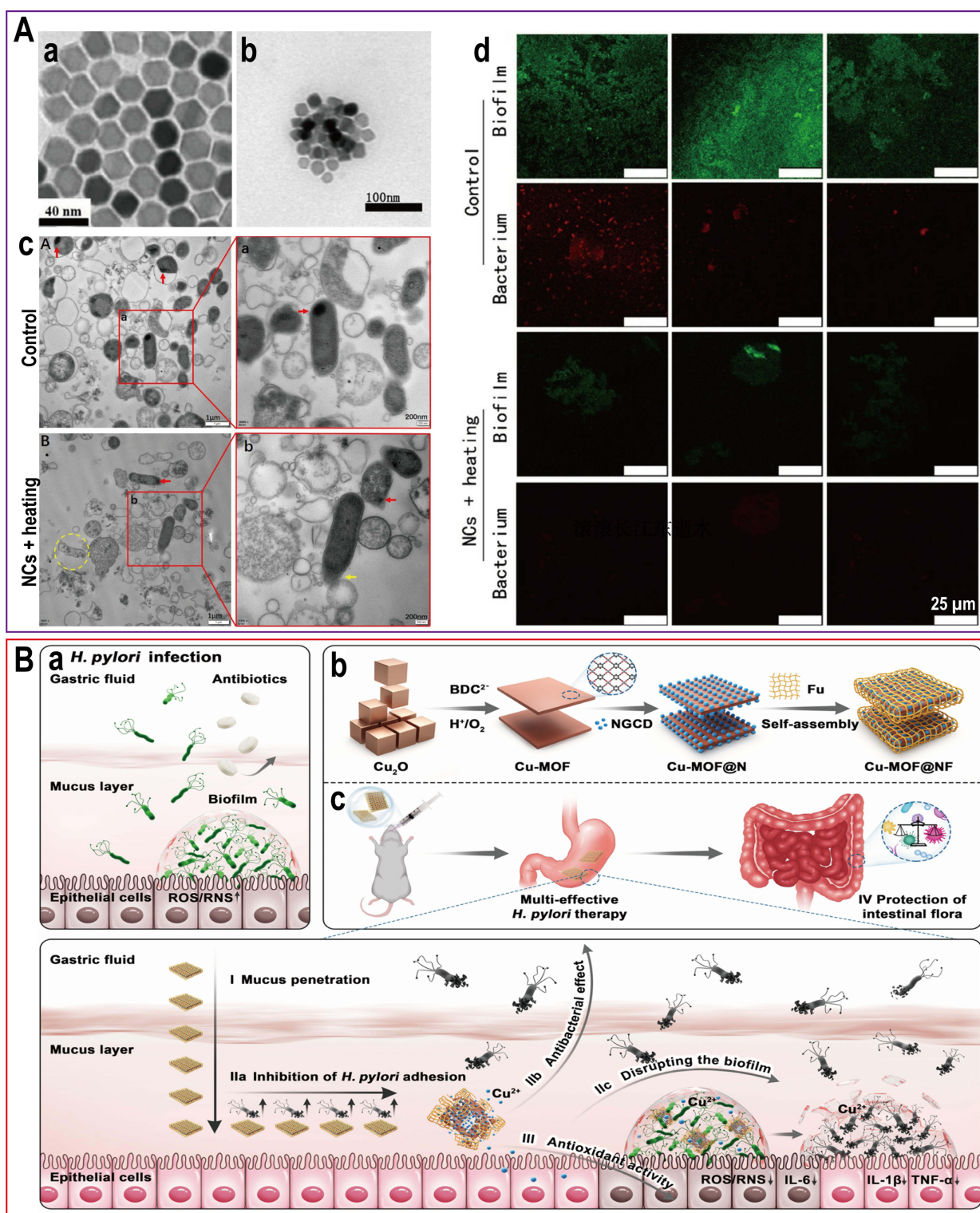
Understanding how nanomaterials engage in *H. pylori* biofilm formation is of great significance for the development of nanomaterials. Based on the aforementioned mechanisms of *H. pylori* biofilm formation, nanomaterials can play a disruptive role at various stages, such as inhibiting biofilm formation, 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  $\text{Zn}_{0.3}\text{Fe}_{2.7}\text{O}_4$  NPs using poly(ethylene glycol)-*b*-poly( $\epsilon$ -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).<sup>150</sup> 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  $\text{Cu}^{2+}$  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).<sup>151</sup> In addition, Camargo et al synthesized silver-containing compounds and loaded them into polymer (poly[ $\epsilon$ -caprolactone]) NPs. This nanomaterial not only inhibited the formation of *H. pylori* biofilm but also had a destructive effect on mature biofilm.<sup>152</sup>

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*.<sup>153</sup> 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.<sup>154</sup>





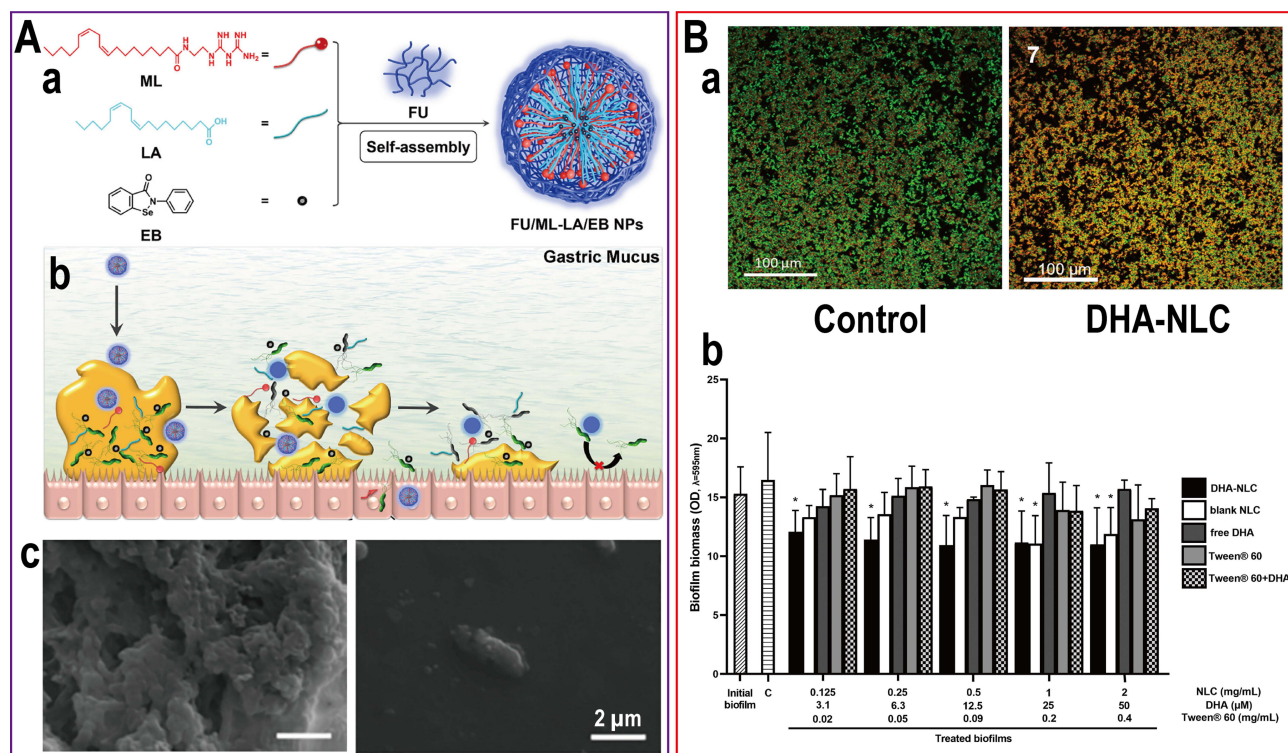
**Figure 3** Nanomaterial-based treatment of *H. pylori* biofilm through inhibition of its formation. **(A)** The suppressive impact of nanoclusters (NCs) on the formation of *H. pylori* biofilm: (a) SEM image of  $\text{ZnO}_3\text{Fe}_{2.7}\text{O}_4$  NPs; (b) TEM image of NCs; (c) photothermal effects 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.<sup>150</sup> **(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 license 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).<sup>151</sup>

## Enhancement of *H. pylori* Biofilm Permeability

Enhancing bacterial biofilm permeability relies mainly on the size, surface modification, surface potential, and other characteristics of nanomaterials.<sup>123,155,156</sup> 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.<sup>36</sup> 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.<sup>157</sup> 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.<sup>158</sup>

## 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.<sup>159–161</sup> 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.<sup>36</sup> **(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 Elsevier.<sup>157</sup>



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 nanoplateform had minimal effect on the intestinal microbiota and contributed to the repair of the gastric mucosa.<sup>159</sup> In addition, Fan et al constructed PtCu<sub>3</sub>-PDA@AIPH@fucoidan (PPAF) NPs capable of generating alkyl radicals (R·). This nanomaterial was composed of PtCu<sub>3</sub>, 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.<sup>160</sup>

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.<sup>35,163–165</sup> Li et al described a multifunctional antibiotic-free copper–organic framework (HKUST-1) platform (Figure 5B), which was

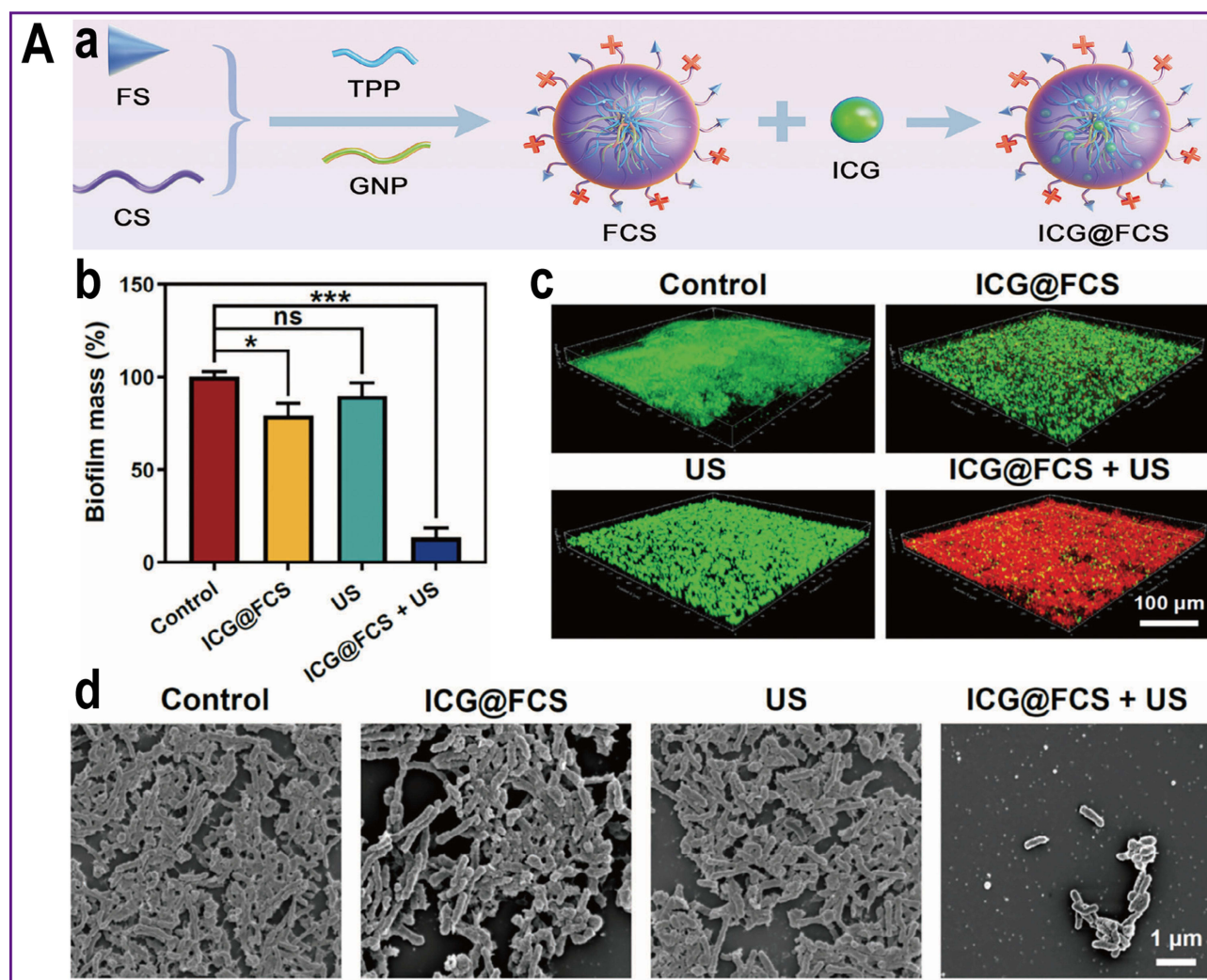
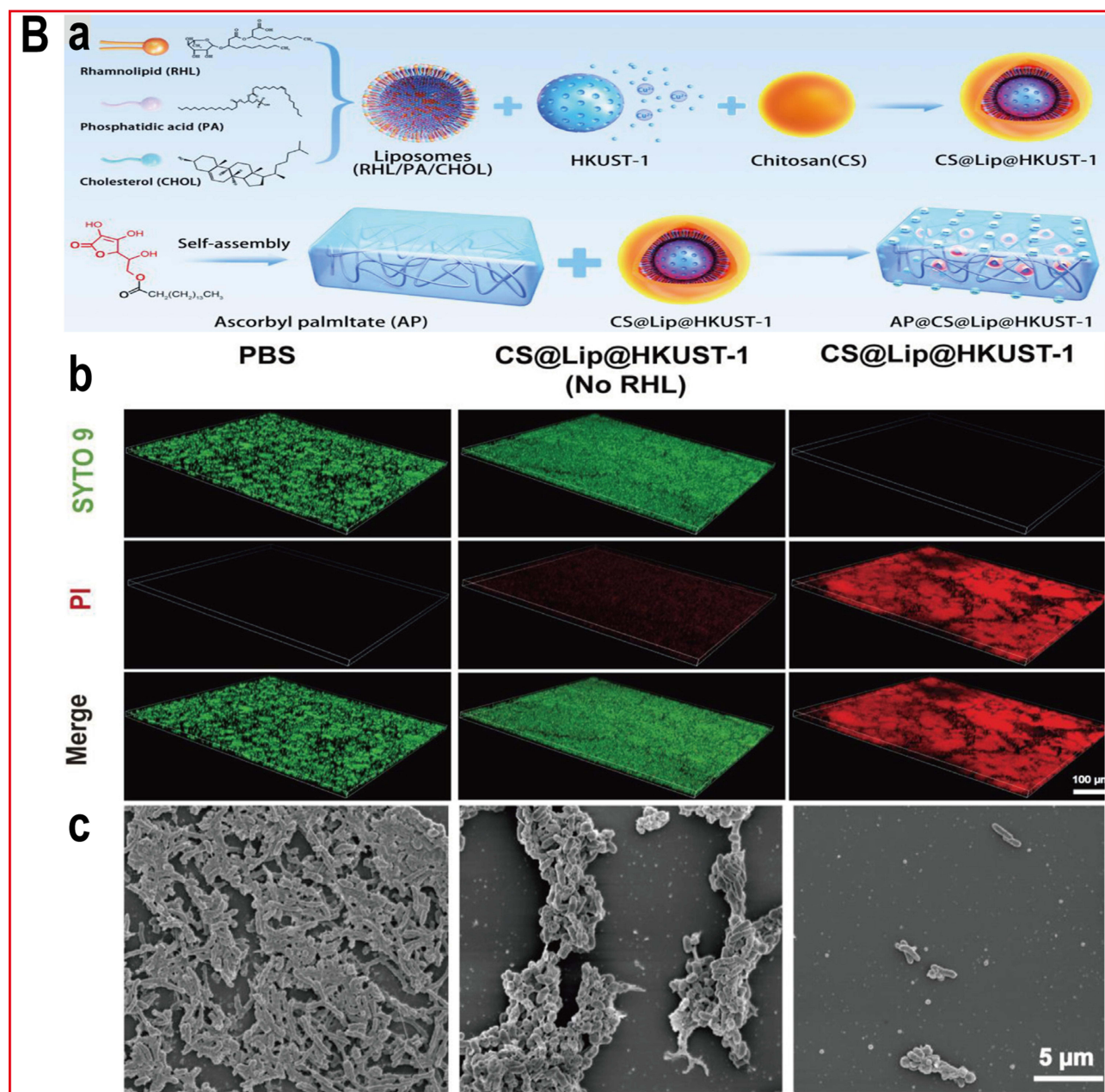


Figure 5 Continued.



**Figure 5** Nanomaterial-based treatment of *H. pylori* biofilm through disruption of mature biofilm. **(A)** Synthesis of  $\text{ICG@FCS}$  and disruption of mature *H. pylori* mature biofilm: (a) synthesis of  $\text{ICG@FCS}$  — detecting the impact of sonodynamic therapy of  $\text{ICG@FCS}$  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 CC BY license.<sup>159</sup> **(B)** Synthesis of  $\text{AP@CS@Lip@HKUST-1}$  and disruption of mature *H. pylori* biofilm: (a) synthesis of  $\text{AP@CS@Lip@HKUST-1}$  — detecting the impact of  $\text{CS@Lip@HKUST-1}$  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.<sup>162</sup>

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  $\text{AP@CS@Lip@HKUST-1}$ . 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.<sup>162</sup>

Phototherapy, including photothermal therapy and photodynamic therapy, is widely applied in the treatment of *H. pylori* biofilm.<sup>37</sup> Qiao et al designed a phototherapy nanomedicine ( $\text{RLs@T780TG}$ ) 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.<sup>166</sup>

## 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.<sup>167</sup> Additionally, drug-delivery systems can exert synergistic effects to enhance antimicrobial efficiency and reduce drug toxicity through precise drug release.<sup>168</sup> 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.<sup>38,44,169,170</sup> 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.<sup>171</sup>

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<sup>2000</sup> 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.<sup>35</sup> 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

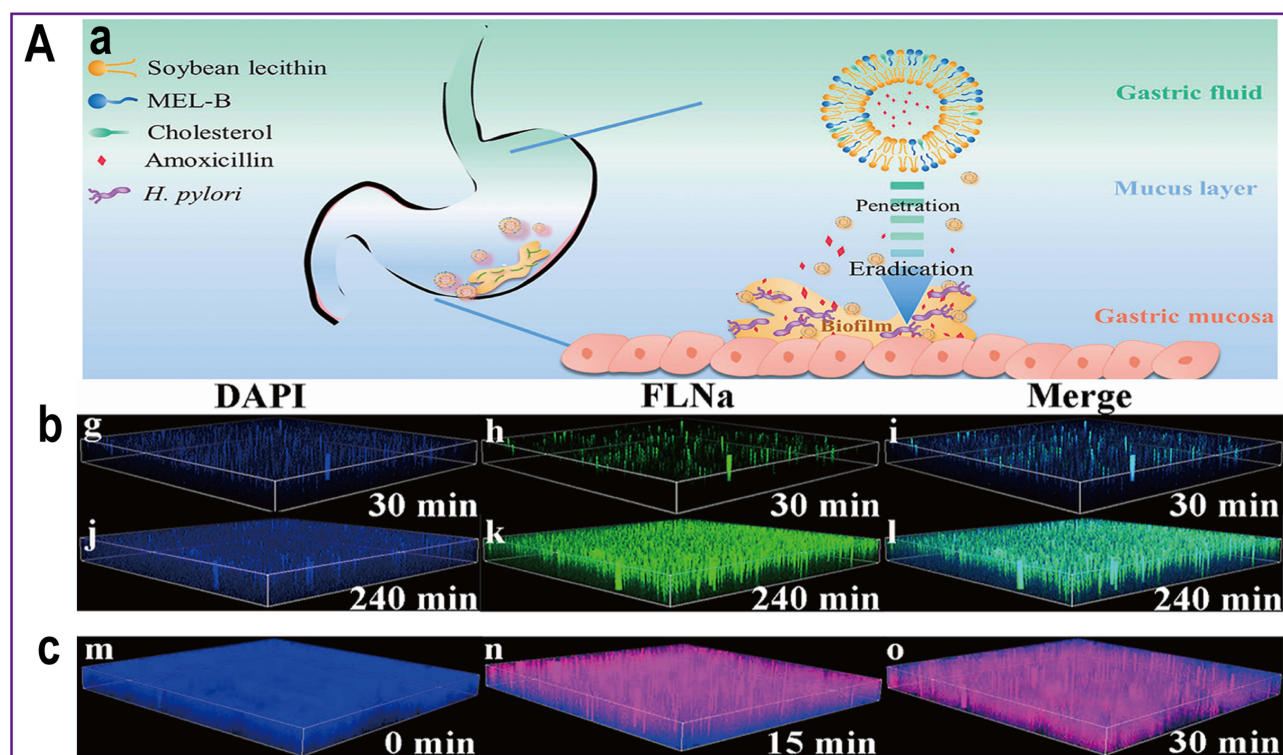
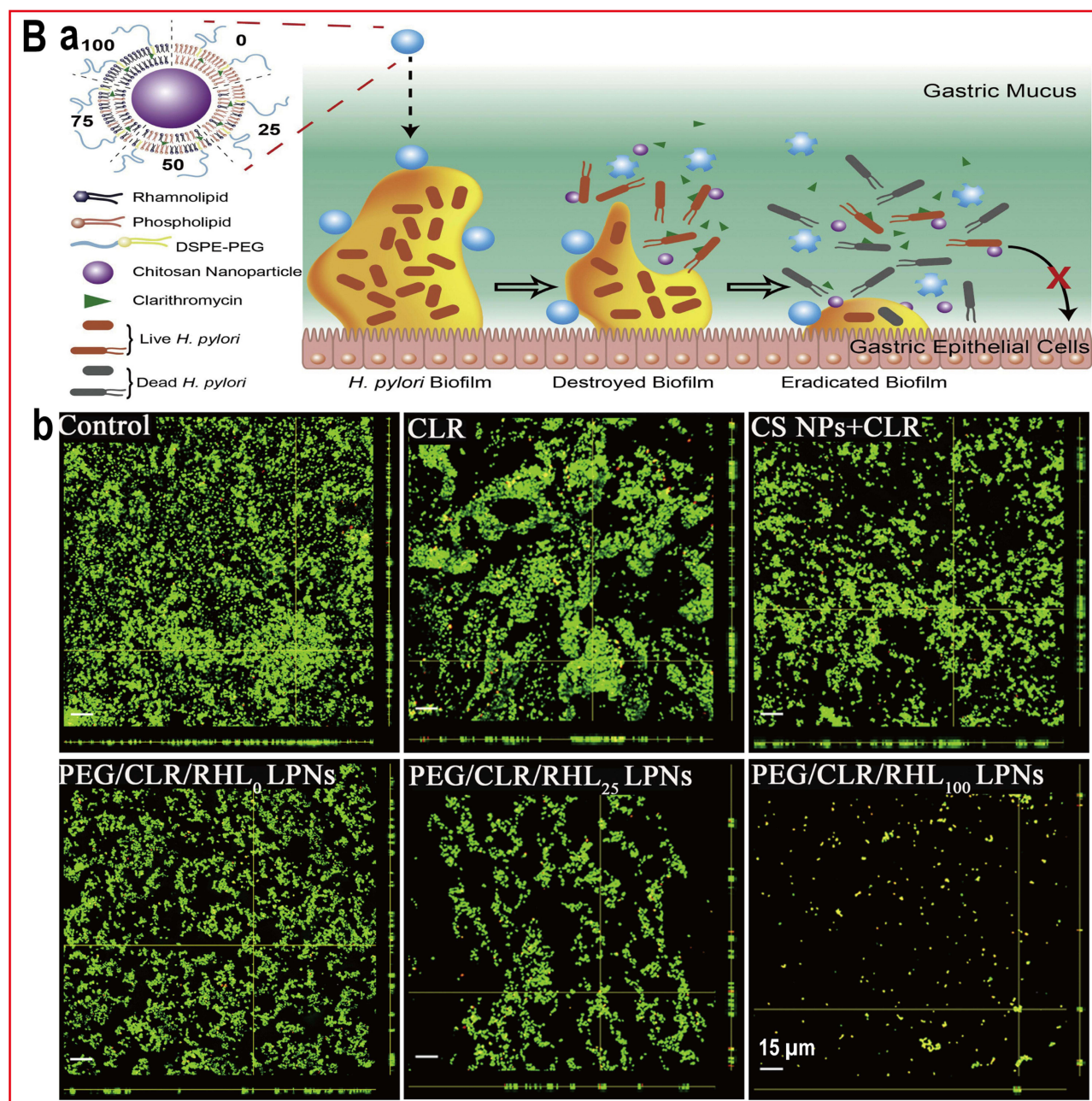


Figure 6 Continued.



**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 Nile red by CLSM. Reproduced from Wu Y, Geng J, Cheng X, et al. Cosmetic-derived mannoseylerythritol 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.<sup>171</sup> **(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.<sup>35</sup>

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.<sup>172</sup>

## 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.<sup>173</sup> 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.<sup>174</sup> Lipid-based NPs have certain shortcomings. Their physicochemical properties are unstable and are easily affected by pH and temperature.<sup>175,176</sup> Furthermore, due to the sensitivity of lipids to high temperatures, special sterilization techniques are required, and completely eliminating pathogens is relatively difficult.<sup>177</sup> 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.<sup>178</sup>

## 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.

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## Disclosure

The authors declare that they have no conflicts of interest.

## References

- Tran SC, Bryant KN, Cover TL. The *Helicobacter pylori* cag pathogenicity island as a determinant of gastric cancer risk. *Gut Microbes*. 2024;16(1):2314201. doi:10.1080/19490976.2024.2314201
- Zhou XZ, Lyu NH, Zhu HY, et al. Large-scale, national, family-based epidemiological study on *Helicobacter pylori* infection in China: the time to change practice for related disease prevention. *Gut*. 2023;72(5):855–869. doi:10.1136/gutjnl-2022-328965
- Li Y, Choi H, Leung K, et al. Global prevalence of *Helicobacter pylori* infection between 1980 and 2022: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. 2023;8(6):553–564. doi:10.1016/s2468-1253(23)00070-5
- Zeng J, Xie C, Huang Z, et al. LOX-I acts as an N(6)-methyladenosine-regulated receptor for *Helicobacter pylori* by binding to the bacterial catalase. *Nat Commun*. 2024;15(1):669. doi:10.1038/s41467-024-44860-9
- Shi L, Shangguan J, Lu Y, et al. ROS-mediated up-regulation of SAE1 by *Helicobacter pylori* promotes human gastric tumor genesis and progression. *J Transl Med*. 2024;22(1):148. doi:10.1186/s12967-024-04913-5
- Zheng H, Zhang T, Zhang J, et al. AUF1-mediated inhibition of autophagic lysosomal degradation contributes to CagA stability and *Helicobacter pylori*-induced inflammation. *Gut Microbes*. 2024;16(1):2382766. doi:10.1080/19490976.2024.2382766
- Meng F, Zhu S, Gong M, et al. Heat shock protein 70 is involved in polaprezinc driven cell protection against *Helicobacter pylori*-induced injury. *Int J Med Microbiol*. 2023;313(3):151582. doi:10.1016/j.ijmm.2023.151582
- Health Nlo Eight substances added to 15th Report on Carcinogens[EB/OL]. Available from: <https://www.nih.gov/news-events/news-releases/eight-substances-added-15th-report-carcinogens>. Accessed April 10, 2025.
- Sun L, Zheng H, Qiu M, et al. *Helicobacter pylori* infection and risk of cardiovascular disease. *Helicobacter*. 2023;28(3):e12967. doi:10.1111/hel.12967
- Queiroz DMM, Braga LLC, Rocha GA, et al. Infection by *Helicobacter pylori* cytotoxin-A-associated antigen-positive strains is associated with iron deficiency anemia in a longitudinal birth cohort in Brazil. *Haematologica*. 2024. doi:10.3324/haematol.2024.285446
- Wang F, Yao Z, Jin T, et al. Research progress on *Helicobacter pylori* infection related neurological diseases. *Ageing Res Rev*. 2024;99:102399. doi:10.1016/j.arr.2024.102399
- O'Connor A, Lamarque D, Gisbert JP, et al. Treatment of *Helicobacter pylori* infection 2017. *Helicobacter*. 2017;22(Suppl 1). doi:10.1111/hel.12410
- Tshibangu-Kabamba E, Yamaoka Y. *Helicobacter pylori* infection and antibiotic resistance - from biology to clinical implications. *Nat Rev Gastroenterol Hepatol*. 2021;18(9):613–629. doi:10.1038/s41575-021-00449-x
- Attaran B, Falsafi T, Ghorbanmehr N. Effect of biofilm formation by clinical isolates of *Helicobacter pylori* on the efflux-mediated resistance to commonly used antibiotics. *World J Gastroenterol*. 2017;23(7):1163–1170. doi:10.3748/wjg.v23.i7.1163
- Wu X, Wu D, Cui G, et al. Association between biofilm formation and structure and antibiotic resistance in *H. pylori*. *Infect Drug Resist*. 2024;17:2501–2512. doi:10.2147/idr.S468126
- Hathroubi S, Zerebinski J, Ottemann KM. *Helicobacter pylori* biofilm involves a multigene stress-biased response, including a structural role for flagella. *mBio*. 2018;9(5). doi:10.1128/mBio.01973-18
- Cai Y, Wang C, Chen Z, et al. Transporters HP0939, HP0497, and HP0471 participate in intrinsic multidrug resistance and biofilm formation in *Helicobacter pylori* by enhancing drug efflux. *Helicobacter*. 2020;25(4):e12715. doi:10.1111/hel.12715
- Zhong Z, Wang X, Li J, et al. A study on the diagnosis of the *Helicobacter pylori* coccoid form with artificial intelligence technology. *Front Microbiol*. 2022;13:1008346. doi:10.3389/fmicb.2022.1008346
- Seal BS, Drider D, Oakley BB, et al. Microbial-derived products as potential new antimicrobials. *Vet Res*. 2018;49(1):66. doi:10.1186/s13567-018-0563-5
- Pletzer D, Hancock RE. Antibiofilm peptides: potential as broad-spectrum agents. *J Bacteriol*. 2016;198(19):2572–2578. doi:10.1128/jb.00017-16
- Yasir M, Willcox MDP, Dutta D. Action of antimicrobial peptides against bacterial biofilms. *Materials*. 2018;11(12). doi:10.3390/ma11122468
- Windham IH, Servetas SL, Whitmire JM, et al. *Helicobacter pylori* biofilm formation is differentially affected by common culture conditions, and proteins play a central role in the biofilm matrix. *Appl Environ Microbiol*. 2018;84(14). doi:10.1128/aem.00391-18
- Salas-Jara MJ, Sanhueza EA, Retamal-Díaz A, et al. Probiotic *Lactobacillus fermentum* UCO-979C biofilm formation on AGS and Caco-2 cells and *Helicobacter pylori* inhibition. *Biofouling*. 2016;32(10):1245–1257. doi:10.1080/08927014.2016.1249367
- Ji J, Yang H. In vitro effects of *Lactobacillus plantarum* LN66 and antibiotics used alone or in combination on *Helicobacter pylori* mature biofilm. *Microorganisms*. 2021;9(2). doi:10.3390/microorganisms9020424
- Di Lodovico S, Napoli E, Di Campli E, et al. Pistacia vera L. oleoresin and levofloxacin is a synergistic combination against resistant *Helicobacter pylori* strains. *Sci Rep*. 2019;9(1):4646. doi:10.1038/s41598-019-40991-y
- Chen Y, Gao Y, Chen Y, et al. Nanomaterials-based photothermal therapy and its potentials in antibacterial treatment. *J Control Release*. 2020;328:251–262. doi:10.1016/j.jconrel.2020.08.055
- Zhang Y, Yue T, Gu W, et al. pH-responsive hierarchical H(2)S-releasing nano-disinfectant with deep-penetrating and anti-inflammatory properties for synergistically enhanced eradication of bacterial biofilms and wound infection. *J Nanobiotechnology*. 2022;20(1):55. doi:10.1186/s12951-022-01262-7
- Finbloom JA, Sousa F, Stevens MM, et al. Engineering the drug carrier biointerface to overcome biological barriers to drug delivery. *Adv Drug Deliv Rev*. 2020;167:89–108. doi:10.1016/j.addr.2020.06.007



29. Makabenta JMV, Nabawy A, Li CH, et al. Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. *Nat Rev Microbiol.* **2021**;19(1):23–36. doi:10.1038/s41579-020-0420-1
30. Lai Y, Wei W, Du Y, et al. Biomaterials for *Helicobacter pylori* therapy: therapeutic potential and future perspectives. *Gut Microbes.* **2022**;14(1):2120747. doi:10.1080/19490976.2022.2120747
31. Barman S, Kurnaz LB, Leighton R, et al. Intrinsic antimicrobial resistance: molecular biomaterials to combat microbial biofilms and bacterial persisters. *Biomaterials.* **2024**;311:122690. doi:10.1016/j.biomaterials.2024.122690
32. Yuan P, Ding X, Yang YY, et al. Metal nanoparticles for diagnosis and therapy of bacterial infection. *Adv Healthc Mater.* **2018**;7(13):e1701392. doi:10.1002/adhm.201701392
33. Tang L, Zhang Z, Ding W, et al. Preparation, characterization, and *Staphylococcus aureus* biofilm elimination effect of baicalein-loaded tyrosine/hyaluronic acid/ $\beta$ -cyclodextrin-grafted chitosan nano-delivery system. *Int J Biol Macromol.* **2024**;254(Pt 3):128066. doi:10.1016/j.ijbiomac.2023.128066
34. Zhang J, Leifer F, Rose S, et al. Amikacin Liposome Inhalation Suspension (ALIS) penetrates non-tuberculous mycobacterial biofilms and enhances Amikacin uptake into macrophages. *Front Microbiol.* **2018**;9:915. doi:10.3389/fmicb.2018.00915
35. 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. doi:10.1016/j.jconrel.2019.02.039
36. 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. doi:10.1016/j.jconrel.2022.05.044
37. Qiao Y, Ma Y, Tong Y, et al. Phototherapy and mechanism exploration of biofilm and multidrug-resistant *Helicobacter pylori* by bacteria-targeted NIR photosensitizer. *Small.* **2023**;19(4):e2205248. doi:10.1002/sml.202205248
38. Yang SJ, Huang CH, Yang JC, et al. Residence time-extended nanoparticles by magnetic field improve the eradication efficiency of *Helicobacter pylori*. *ACS Appl Mater Interfaces.* **2020**;12(49):54316–54327. doi:10.1021/acsami.0c13101
39. de Souza MPC, de Camargo BAF, Spósito L, et al. Highlighting the use of micro and nanoparticles based-drug delivery systems for the treatment of *Helicobacter pylori* infections. *Crit Rev Microbiol.* **2021**;47(4):435–460. doi:10.1080/1040841x.2021.1895721
40. Wang J, Jiang Z, Wei Y, et al. Multiplexed identification of bacterial biofilm infections based on machine-learning-aided lanthanide encoding. *ACS Nano.* **2022**;16(2):3300–3310. doi:10.1021/acsnano.1c11333
41. Flemming HC, van Hullebusch ED, Neu TR, et al. The biofilm matrix: multitasking in a shared space. *Nat Rev Microbiol.* **2023**;21(2):70–86. doi:10.1038/s41579-022-00791-0
42. Flemming HC, Wingender J, Szewzyk U, et al. Biofilms: an emergent form of bacterial life. *Nat Rev Microbiol.* **2016**;14(9):563–575. doi:10.1038/nrmicro.2016.94
43. 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. doi:10.1016/j.micres.2023.127539
44. 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**. doi:10.1016/j.ejpb.2023.10.021
45. 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. doi:10.1016/j.gassur.2005.10.019
46. Hou C, Yin F, Wang S, et al. *Helicobacter pylori* biofilm-related drug resistance and new developments in its anti-biofilm agents. *Infect Drug Resist.* **2022**;15:1561–1571. doi:10.2147/idr.S357473
47. Hathroubi S, Servetas SL, Windham I, et al. *Helicobacter pylori* biofilm formation and its potential role in pathogenesis. *Microbiol Mol Biol Rev.* **2018**;82(2). doi:10.1128/mmb.00001-18
48. Whitfield C, Wear SS, Sande C. Assembly of bacterial capsular polysaccharides and exopolysaccharides. *Annu Rev Microbiol.* **2020**;74:521–543. doi:10.1146/annurev-micro-011420-075607
49. Wang X, Zhang X, Wang Y, et al. Yeast cell wall polysaccharides in Tibetan kefir grains are key substances promoting the formation of bacterial biofilm. *Carbohydr Polym.* **2023**;300:120247. doi:10.1016/j.carbpol.2022.120247
50. Stark RM, Gerwig GJ, Pitman RS, et al. Biofilm formation by *Helicobacter pylori*. *Lett Appl Microbiol.* **1999**;28(2):121–126. doi:10.1046/j.1365-2672.1999.00481.x
51. Yan W, Qian T, Zhang L, et al. Interaction of perfluorooctanoic acid with extracellular polymeric substances - Role of protein. *J Hazard Mater.* **2021**;401:123381. doi:10.1016/j.jhazmat.2020.123381
52. Hathroubi S, Mekni MA, Domenico P, et al. Biofilms: microbial shelters against antibiotics. *Microb Drug Resist.* **2017**;23(2):147–156. doi:10.1089/mdr.2016.0087
53. Jakubovics NS, Burgess JG. Extracellular DNA in oral microbial biofilms. *Microbes Infect.* **2015**;17(7):531–537. doi:10.1016/j.micinf.2015.03.015
54. Grande R, Di Giulio M, Bessa LJ, et al. Extracellular DNA in *Helicobacter pylori* biofilm: a backstairs rumour. *J Appl Microbiol.* **2011**;110(2):490–498. doi:10.1111/j.1365-2672.2010.04911.x
55. Grande R, Di Marcantonio MC, Robuffo I, et al. *Helicobacter pylori* ATCC 43629/NCTC 11639 Outer Membrane Vesicles (OMVs) from biofilm and planktonic phase associated with extracellular DNA (eDNA). *Front Microbiol.* **2015**;6:1369. doi:10.3389/fmicb.2015.01369
56. Mashburn-Warren LM, Whiteley M. Special delivery: vesicle trafficking in prokaryotes. *Mol Microbiol.* **2006**;61(4):839–846. doi:10.1111/j.1365-2958.2006.05272.x
57. Wang W, Chanda W, Zhong M. The relationship between biofilm and outer membrane vesicles: a novel therapy overview. *FEMS Microbiol Lett.* **2015**;362(15):fnv117. doi:10.1093/femsle/fnv117
58. Yonezawa H, Osaki T, Kurata S, et al. Outer membrane vesicles of *Helicobacter pylori* TK1402 are involved in biofilm formation. *BMC Microbiol.* **2009**;9:197. doi:10.1186/1471-2180-9-197
59. Yonezawa H, Osaki T, Woo T, et al. Analysis of outer membrane vesicle protein involved in biofilm formation of *Helicobacter pylori*. *Anaerobe.* **2011**;17(6):388–390. doi:10.1016/j.anaerobe.2011.03.020
60. Fauzia KA, Effendi WI, Alfaray RI, et al. Molecular mechanisms of biofilm formation in *Helicobacter pylori*. *Antibiotics.* **2024**;13(10). doi:10.3390/antibiotics13100976

61. Ratthawongjirakul P, Thongkerd V, Chaicumpa W. The impacts of a fliD mutation on the biofilm formation of *Helicobacter pylori*. *Asian Pac J Trop Biomed*. 2016;6(12):1008–1014. doi:10.1016/j.apjtb.2016.10.005
62. Wong EH, Ng CG, Chua EG, et al. Comparative genomics revealed multiple *Helicobacter pylori* genes associated with biofilm formation in vitro. *PLoS One*. 2016;11(11):e0166835. doi:10.1371/journal.pone.0166835
63. Azevedo NF, Pinto AR, Reis NM, et al. Shear stress, temperature, and inoculation concentration influence the adhesion of water-stressed *Helicobacter pylori* to stainless steel 304 and polypropylene. *Appl Environ Microbiol*. 2006;72(4):2936–2941. doi:10.1128/AEM.72.4.2936-2941.2006
64. Hathroubi S, Hu S, Ottemann KM. Genetic requirements and transcriptomics of *Helicobacter pylori* biofilm formation on abiotic and biotic surfaces. *NPJ Biofilms Microbiomes*. 2020;6(1):56. doi:10.1038/s41522-020-00167-3
65. Gu H. Role of Flagella in the pathogenesis of *Helicobacter pylori*. *Curr Microbiol*. 2017;74(7):863–869. doi:10.1007/s00284-017-1256-4
66. Williams JC, McInnis KA, Testerman TL. Adherence of *Helicobacter pylori* to abiotic surfaces is influenced by serum. *Appl Environ Microbiol*. 2008;74(4):1255–1258. doi:10.1128/aem.01958-07
67. Yonezawa H, Osaki T, Fukutomi T, et al. Diversification of the AlpB outer membrane protein of *Helicobacter pylori* affects biofilm formation and cellular adhesion. *J Bacteriol*. 2017;199(6). doi:10.1128/jb.00729-16
68. Cole SP, Harwood J, Lee R, et al. Characterization of monospecies biofilm formation by *Helicobacter pylori*. *J Bacteriol*. 2004;186(10):3124–3132. doi:10.1128/jb.186.10.3124-3132.2004
69. Percival SL, Thomas JG. Transmission of *Helicobacter pylori* and the role of water and biofilms. *J Water Health*. 2009;7(3):469–477. doi:10.2166/wh.2009.070
70. Servetas SL, Kim A, Su H, et al. Comparative analysis of the Hom family of outer membrane proteins in isolates from two geographically distinct regions: the United States and South Korea. *Helicobacter*. 2018;23(2):e12461. doi:10.1111/hel.12461
71. Hall MR, McGillicuddy E, Kaplan LJ. Biofilm: basic principles, pathophysiology, and implications for clinicians. *Surg Infect*. 2014;15(1):1–7. doi:10.1089/sur.2012.129
72. Anderson JK, Huang JY, Wreden C, et al. Chemorepulsion from the Quorum signal Autoinducer-2 promotes *Helicobacter pylori* biofilm dispersal. *mBio*. 2015;6(4):e00379. doi:10.1128/mBio.00379-15
73. Krzyżek P, Migdał P, Paluch E, et al. Myricetin as an antivirulence compound interfering with a morphological transformation into coccoid forms and potentiating activity of antibiotics against *Helicobacter pylori*. *Int J Mol Sci*. 2021;22(5). doi:10.3390/ijms22052695
74. Krzyżek P, Migdał P, Grande R, et al. Biofilm formation of *Helicobacter pylori* in both static and microfluidic conditions is associated with resistance to clarithromycin. *Front Cell Infect Microbiol*. 2022;12:868905. doi:10.3389/fcimb.2022.868905
75. Luo P, Huang Y, Hang X, et al. Dihydrotanshinone I is effective against drug-resistant *Helicobacter pylori* in vitro and in vivo. *Antimicrob Agents Chemother*. 2021;65(3). doi:10.1128/aac.01921-20
76. Bugli F, Palmieri V, Torelli R, et al. In vitro effect of clarithromycin and alginate lyase against *Helicobacter pylori* biofilm. *Biotechnol Prog*. 2016;32(6):1584–1591. doi:10.1002/btpr.2339
77. Kadhodaei S, Siavoshi F, Akbari Noghabi K. Mucoid and coccoid *Helicobacter pylori* with fast growth and antibiotic resistance. *Helicobacter*. 2020;25(2):e12678. doi:10.1111/hel.12678
78. El Mortaji L, Tejada-Arranz A, Rifflet A, et al. A peptide of a type I toxin-antitoxin system induces *Helicobacter pylori* morphological transformation from spiral shape to coccoids. *Proc Natl Acad Sci U S A*. 2020;117(49):31398–31409. doi:10.1073/pnas.2016195117
79. Solano C, Echeverez M, Lasa I. Biofilm dispersion and quorum sensing. *Curr Opin Microbiol*. 2014;18:96–104. doi:10.1016/j.mib.2014.02.008
80. Lee WK, Ogura K, Loh JT, et al. Quantitative effect of luxS gene inactivation on the fitness of *Helicobacter pylori*. *Appl Environ Microbiol*. 2006;72(10):6615–6622. doi:10.1128/aem.01291-06
81. Mah TF. Biofilm-specific antibiotic resistance. *Future Microbiol*. 2012;7(9):1061–1072. doi:10.2217/fmb.12.76
82. Rabin N, Zheng Y, Opoku-Temeng C, et al. Biofilm formation mechanisms and targets for developing antibiofilm agents. *Future Med Chem*. 2015;7(4):493–512. doi:10.4155/fmc.15.6
83. Fauzia KA, Miftahussurur M, Syam AF, et al. Biofilm formation and antibiotic resistance phenotype of *Helicobacter pylori* clinical isolates. *Toxins*. 2020;12(8). doi:10.3390/toxins12080473
84. Penesyan A, Paulsen IT, Gillings MR, et al. Secondary effects of antibiotics on microbial biofilms. *Front Microbiol*. 2020;11:2109. doi:10.3389/fmicb.2020.02109
85. Tseng BS, Zhang W, Harrison JJ, et al. The extracellular matrix protects *Pseudomonas aeruginosa* biofilms by limiting the penetration of tobramycin. *Environ Microbiol*. 2013;15(10):2865–2878. doi:10.1111/1462-2920.12155
86. Hall CW, Mah TF. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiol Rev*. 2017;41(3):276–301. doi:10.1093/femsre/flux010
87. Tseng YS, Wu DC, Chang CY, et al. Amoxicillin resistance with beta-lactamase production in *Helicobacter pylori*. *Eur J Clin Invest*. 2009;39(9):807–812. doi:10.1111/j.1365-2362.2009.02166.x
88. Harris AG, Hazell SL, Netting AG. Use of digoxigenin-labelled ampicillin in the identification of penicillin-binding proteins in *Helicobacter pylori*. *J Antimicrob Chemother*. 2000;45(5):591–598. doi:10.1093/jac/45.5.591
89. Reshetnyak VI, Reshetnyak TM. Significance of dormant forms of *Helicobacter pylori* in ulcerogenesis. *World J Gastroenterol*. 2017;23(27):4867–4878. doi:10.3748/wjg.v23.i27.4867
90. Costa K, Bacher G, Allmaier G, et al. The morphological transition of *Helicobacter pylori* cells from spiral to coccoid is preceded by a substantial modification of the cell wall. *J Bacteriol*. 1999;181(12):3710–3715. doi:10.1128/jb.181.12.3710-3715.1999
91. Kuo CJ, Guo RT, Lu IL, et al. Crystal structures and computer screened inhibitors of *Helicobacter pylori* undecaprenyl pyrophosphate synthase. In: 2007 Frontiers in the Convergence of Bioscience and Information Technologies, 11–13 October 2007; 2007:305–310.
92. Modak JK, Tikhomirova A, Gorrell RJ, et al. Anti-*Helicobacter pylori* activity of ethoxzolamide. *J Enzyme Inhib Med Chem*. 2019;34(1):1660–1667. doi:10.1080/14756366.2019.1663416
93. Wang G, Maier SE, Lo LF, et al. Peptidoglycan deacetylation in *Helicobacter pylori* contributes to bacterial survival by mitigating host immune responses. *Infect Immun*. 2010;78(11):4660–4666. doi:10.1128/iai.00307-10
94. Zhao Y, Cai Y, Chen Z, et al. SpoT-mediated NapA upregulation promotes oxidative stress-induced *Helicobacter pylori* biofilm formation and confers multidrug resistance. *Antimicrob Agents Chemother*. 2023;65(5). doi:10.1128/aac.00152-21

95. Chou FP, Tsai CT, Chiou YS, et al. An enzymatic approach to configurationally rare trans-androsteronyl- $\alpha$ -glucoside and Its potential anticancer application. *Chem Biol Drug Des.* **2017**;89(1):61–66. doi:10.1111/cbdd.12830
96. Qaria MA, Kumar N, Hussain A, et al. Roles of cholesteryl- $\alpha$ -glucoside transferase and cholesteryl glucosides in maintenance of *Helicobacter pylori* morphology, cell wall integrity, and resistance to antibiotics. *mBio.* **2018**;9(6). doi:10.1128/mBio.01523-18
97. Raj DS, Kumar Kesavan D, Muthusamy N, et al. Efflux pumps potential drug targets to circumvent drug resistance – multi drug efflux pumps of *Helicobacter pylori*. *Mater Today Proc.* **2021**;45:2976–2981. doi:10.1016/j.matpr.2020.11.955
98. Krzyżek P, Grande R, Migdał P, et al. Biofilm formation as a complex result of virulence and adaptive responses of *Helicobacter pylori*. *Pathogens.* **2020**;9(12). doi:10.3390/pathogens9121062
99. Yonezawa H, Osaki T, Hojo F, et al. Effect of *Helicobacter pylori* biofilm formation on susceptibility to amoxicillin, metronidazole and clarithromycin. *Microb Pathog.* **2019**;132:100–108. doi:10.1016/j.micpath.2019.04.030
100. Ge X, Cai Y, Chen Z, et al. Bifunctional enzyme SpoT is involved in biofilm formation of *Helicobacter pylori* with multidrug resistance by upregulating efflux pump Hp1174 (gluP). *Antimicrob Agents Chemother.* **2018**;62(11). doi:10.1128/aac.00957-18
101. Marcus EA, Inatomi N, Nagami GT, et al. The effects of varying acidity on *Helicobacter pylori* growth and the bactericidal efficacy of ampicillin. *Aliment Pharmacol Ther.* **2012**;36(10):972–979. doi:10.1111/apt.12059
102. Wong EHJ, Ng CG, Goh KL, et al. Metabolomic analysis of low and high biofilm-forming *Helicobacter pylori* strains. *Sci Rep.* **2018**;8(1):1409. doi:10.1038/s41598-018-19697-0
103. Njume C, Afolayan AJ, Samie A, et al. Inhibitory and bactericidal potential of crude acetone extracts of *Combretum molle* (Combretaceae) on drug-resistant strains of *Helicobacter pylori*. *J Health Popul Nutr.* **2011**;29(5):438–445. doi:10.3329/jhpn.v29i5.8897
104. Yonezawa H, Osaki T, Hanawa T, et al. Impact of *Helicobacter pylori* biofilm formation on clarithromycin susceptibility and generation of resistance mutations. *PLoS One.* **2013**;8(9):e73301. doi:10.1371/journal.pone.0073301
105. Michaelis C, Grohmann E. Horizontal gene transfer of antibiotic resistance genes in biofilms. *Antibiotics.* **2023**;12(2). doi:10.3390/antibiotics12020328
106. Okshevsky M, Meyer RL. The role of extracellular DNA in the establishment, maintenance and perpetuation of bacterial biofilms. *Crit Rev Microbiol.* **2015**;41(3):341–352. doi:10.3109/1040841x.2013.841639
107. Campoccia D, Montanaro L, Arciola CR. Extracellular DNA (eDNA). A major ubiquitous element of the bacterial biofilm architecture. *Int J Mol Sci.* **2021**;22(16). doi:10.3390/ijms22169100
108. Haddadi MH, Khoshnood S, Koupaei M, et al. Evaluating the incidence of ampC- $\beta$ -lactamase genes, biofilm formation, and antibiotic resistance among hypervirulent and classical *Klebsiella pneumoniae* strains. *J Appl Microbiol.* **2023**;134(11). doi:10.1093/jambio/txad241
109. Yeh JA, Huang HK, Chou AL, et al. *Helicobacter pylori* eradication with high-dose proton pump inhibitor-amoxicillin dual therapy: a systematic review and meta-analysis. *Int J Antimicrob Agents.* **2024**;63(6):107159. doi:10.1016/j.ijantimicag.2024.107159
110. Jy X, Dong H, Liao L, et al. Deoxyshikonin: a promising lead drug against drug resistance or sensitivity to *Helicobacter pylori* in an acidic environment. *Antimicrob Agents Chemother.* **2024**;68(10):e0095924. doi:10.1128/aac.00959-24
111. Moss SF, Shah SC, Tan MC, et al. Evolving concepts in *Helicobacter pylori* Management. *Gastroenterology.* **2024**;166(2):267–283. doi:10.1053/j.gastro.2023.09.047
112. Hong TC, El-Omar EM, Kuo YT, et al. Primary antibiotic resistance of *Helicobacter pylori* in the Asia-Pacific region between 1990 and 2022: an updated systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* **2024**;9(1):56–67. doi:10.1016/s2468-1253(23)00281-9
113. Xie J, Peng J, Liu D, et al. Treatment failure is a key factor in the development of *Helicobacter pylori* resistance. *Helicobacter.* **2024**;29(3):e13091. doi:10.1111/hel.13091
114. Xia X, Yin Z, Yang Y, et al. In situ upregulating heat shock protein 70 via gastric nano-heaters for the interference of *Helicobacter pylori* Infection. *ACS Nano.* **2022**;16(9):14043–14054. doi:10.1021/acsnano.2c03911
115. Chen CC, Liou JM, Lee YC, et al. The interplay between *Helicobacter pylori* and gastrointestinal microbiota. *Gut Microbes.* **2021**;13(1):1–22. doi:10.1080/19490976.2021.1909459
116. Engelsberger V, Gerhard M, Mejias-Luque R. Effects of *Helicobacter pylori* infection on intestinal microbiota, immunity and colorectal cancer risk. *Front Cell Infect Microbiol.* **2024**;14:1339750. doi:10.3389/fcimb.2024.1339750
117. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol.* **2021**;19(1):55–71. doi:10.1038/s41579-020-0433-9
118. Dickson I. Gut microbiota: diagnosing IBD with the gut microbiome. *Nat Rev Gastroenterol Hepatol.* **2017**;14(4):195. doi:10.1038/nrgastro.2017.25
119. Saravanan J, Nair A, Krishna SS, et al. Nanomaterials in biology and medicine: a new perspective on its toxicity and applications. *Drug Chem Toxicol.* **2024**;47(5):767–784. doi:10.1080/01480545.2024.2340002
120. Patra JK, Das G, Fraceto LF, et al. Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnology.* **2018**;16(1):71. doi:10.1186/s12951-018-0392-8
121. Li SR, Tao SY, Li Q, et al. Harnessing nanomaterials for copper-induced cell death. *Biomaterials.* **2025**;313:122805. doi:10.1016/j.biomaterials.2024.122805
122. Li B, Mao J, Wu J, et al. Nano-Bio Interactions: biofilm-targeted antibacterial nanomaterials. *Small.* **2024**;20(7):e2306135. doi:10.1002/smll.202306135
123. Wang X, Wang D, Lu H, et al. Strategies to promote the journey of nanoparticles against biofilm-associated infections. *Small.* **2024**;20(10):e2305988. doi:10.1002/smll.202305988
124. Patil-Sen Y. Advances in nano-biomaterials and their applications in biomedicine. *Emerg Top Life Sci.* **2021**;5(1):169–176. doi:10.1042/etls20200333
125. Narayana S, Gowda BHH, Hani U, et al. Inorganic nanoparticle-based treatment approaches for colorectal cancer: recent advancements and challenges. *J Nanobiotechnology.* **2024**;22(1):427. doi:10.1186/s12951-024-02701-3
126. Li H, Wang R, Sun H. Systems approaches for unveiling the mechanism of action of Bismuth drugs: new medicinal applications beyond *Helicobacter Pylori* infection. *Acc Chem Res.* **2019**;52(1):216–227. doi:10.1021/acs.accounts.8b00439
127. Bruggaber SF, French G, Thompson RP, et al. Selective and effective bactericidal activity of the cobalt (II) cation against *Helicobacter pylori*. *Helicobacter.* **2004**;9(5):422–428. doi:10.1111/j.1083-4389.2004.00264.x

128. Liu C, Chen S, Sun C, et al. Protonated charge reversal nanodrugs for active targeting clearance of *Helicobacter Pylori* accompanied by gut microbiota protection. *Adv Funct Mater.* **2023**;33(35):2300682.
129. Ivask A, Elbadawy A, Kaweeteerawat C, et al. Toxicity mechanisms in *Escherichia coli* vary for silver nanoparticles and differ from ionic silver. *ACS Nano.* **2014**;8(1):374–386. doi:10.1021/nm4044047
130. Ezraty B, Gennaris A, Barras F, et al. Oxidative stress, protein damage and repair in bacteria. *Nat Rev Microbiol.* **2017**;15(7):385–396. doi:10.1038/nrmicro.2017.26
131. Zhi X, Liu Y, Lin L, et al. Oral pH sensitive GNS@ab nanoprobes for targeted therapy of *Helicobacter pylori* without disturbance gut microbiome. *Nanomedicine.* **2019**;20:102019. doi:10.1016/j.nano.2019.102019
132. Im BN, Shin H, Lim B, et al. *Helicobacter pylori*-targeting multiligand photosensitizer for effective antibacterial endoscopic photodynamic therapy. *Biomaterials.* **2021**;271:120745. doi:10.1016/j.biomaterials.2021.120745
133. Khan I, Saeed K, Khan I. Nanoparticles: properties, applications and toxicities. *Arabian J Chem.* **2019**;12(7):908–931. doi:10.1016/j.arabjc.2017.05.011
134. Musielak E, Feliczak-Guzik A, Nowak I. Synthesis and potential applications of lipid nanoparticles in medicine. *Materials.* **2022**;15(2). doi:10.3390/ma15020682
135. Motsoene F, Abrahamse H, Dhilip Kumar SS. Multifunctional lipid-based nanoparticles for wound healing and antibacterial applications: a review. *Adv Colloid Interface Sci.* **2023**;321:103002. doi:10.1016/j.cis.2023.103002
136. Ferreira M, Pinto SN, Aires-da-silva F, et al. Liposomes as a nanopatform to improve the delivery of antibiotics into *Staphylococcus aureus* biofilms. *Pharmaceutics.* **2021**;13(3). doi:10.3390/pharmaceutics13030321
137. Rukavina Z, Vanić Ž. Current trends in development of liposomes for targeting bacterial biofilms. *Pharmaceutics.* **2016**;8(2). doi:10.3390/pharmaceutics8020018
138. Drulis-Kawa Z, Dorotkiewicz-Jach A, Gubernator J, et al. The interaction between *Pseudomonas aeruginosa* cells and cationic PC:Chol:DOTAP liposomal vesicles versus outer-membrane structure and envelope properties of bacterial cell. *Int J Pharm.* **2009**;367(1–2):211–219. doi:10.1016/j.ijpharm.2008.09.043
139. Martin C, Low WL, Gupta A, et al. Strategies for antimicrobial drug delivery to biofilm. *Curr Pharm Des.* **2015**;21(1):43–66. doi:10.2174/1381612820666140905123529
140. Tenchov R, Bird R, Curtze AE, et al. Lipid nanoparticles—from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. *ACS Nano.* **2021**;15(11):16982–17015. doi:10.1021/acsnano.1c04996
141. Eleraky NE, Allam A, Hassan SB, et al. Nanomedicine fight against antibacterial resistance: an overview of the recent pharmaceutical innovations. *Pharmaceutics.* **2020**;12(2). doi:10.3390/pharmaceutics12020142
142. Borandeh S, van Bochove B, Teotia A, et al. Polymeric drug delivery systems by additive manufacturing. *Adv Drug Deliv Rev.* **2021**;173:349–373. doi:10.1016/j.addr.2021.03.022
143. Beach MA, Nayanathara U, Gao Y, et al. Polymeric nanoparticles for drug delivery. *Chem Rev.* **2024**;124(9):5505–5616. doi:10.1021/acs.chemrev.3c00705
144. Wilczewska AZ, Niemirowicz K, Markiewicz KH, et al. Nanoparticles as drug delivery systems. *Pharmacol Rep.* **2012**;64(5):1020–1037. doi:10.1016/s1734-1140(12)70901-5
145. Liu Y, Shi L, Su L, et al. Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. *Chem Soc Rev.* **2019**;48(2):428–446. doi:10.1039/c7cs00807d
146. Cheung RC, Ng TB, Wong JH, et al. Chitosan: an update on potential biomedical and pharmaceutical applications. *Mar Drugs.* **2015**;13(8):5156–5186. doi:10.3390/md13085156
147. Tao F, Ma S, Tao H, et al. Chitosan-based drug delivery systems: from synthesis strategy to osteomyelitis treatment - A review. *Carbohydr Polym.* **2021**;251:117063. doi:10.1016/j.carbpol.2020.117063
148. Liu Y, Jiang Y, Zhu J, et al. Inhibition of bacterial adhesion and biofilm formation of sulfonated chitosan against *Pseudomonas aeruginosa*. *Carbohydr Polym.* **2019**;206:412–419. doi:10.1016/j.carbpol.2018.11.015
149. Arif M, Sharaf M, Samreen, et al. Chitosan-based nanoparticles as delivery-carrier for promising antimicrobial glycolipid biosurfactant to improve the eradication rate of *Helicobacter pylori* biofilm. *J Biomater Sci Polym Ed.* **2021**;32(6):813–832. doi:10.1080/09205063.2020.1870323
150. 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. doi:10.1186/s12645-022-00121-2
151. 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. doi:10.34133/research.0358
152. Almeida Furquim de Camargo B, Soares Silva DE, Noronha da Silva A, et al. New Silver(I) coordination compound loaded into polymeric nanoparticles as a strategy to improve in vitro anti-*Helicobacter pylori* activity. *Mol Pharm.* **2020**;17(7):2287–2298. doi:10.1021/acs.molpharmaceut.9b01264
153. Zhang Y, Chen Y, Lo C, et al. Inhibition of pathogen adhesion by bacterial outer membrane-coated nanoparticles. *Angew Chem Int Ed Engl.* **2019**;58(33):11404–11408. doi:10.1002/anie.201906280
154. Arif M, Ahmad R, Sharaf M, et al. Antibacterial and antibiofilm activity of mannose-modified chitosan/PMLA nanoparticles against multidrug-resistant *Helicobacter pylori*. *Int J Biol Macromol.* **2022**;223(Pt A):418–432. doi:10.1016/j.ijbiomac.2022.10.265
155. Spósito L, Fonseca D, Gonçalves Carvalho S, et al. Engineering resveratrol-loaded chitosan nanoparticles for potential use against *Helicobacter pylori* infection. *Eur J Pharm Biopharm.* **2024**;199:114280. doi:10.1016/j.ejpb.2024.114280
156. Niaz T, Ihsan A, Abbasi R, et al. Chitosan-albumin based core shell-Corona nano-antimicrobials to eradicate resistant gastric pathogen. *Int J Biol Macromol.* **2019**;138:1006–1018. doi:10.1016/j.ijbiomac.2019.07.165
157. 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. doi:10.1016/j.jconrel.2022.05.050
158. Chen X, Zou Y, Zhang S, et al. Multi-functional vesicles improve *Helicobacter pylori* eradication by a comprehensive strategy based on complex pathological microenvironment. *Acta Pharm Sin B.* **2022**;12(9):3498–3512. doi:10.1016/j.apsb.2022.05.014



159. Yin X, Lai Y, Zhang X, et al. Targeted sonodynamic therapy platform for holistic integrative *Helicobacter pylori* therapy. *Adv Sci*. 2024; e2408583. doi:10.1002/advs.202408583
160. Fan J, Dong Y, Sun Y, et al. Mucus and biofilm penetrating nanoplatform as an ultrasound-induced free radical initiator for targeted treatment of *Helicobacter pylori* infection. *Adv Healthc Mater*. 2024;13(20):e2400363. doi:10.1002/adhm.202400363
161. Yu J, Guo Z, Yan J, et al. Gastric acid-responsive ROS nanogenerators for effective treatment of *Helicobacter pylori* infection without disrupting homeostasis of intestinal flora. *Adv Sci*. 2023;10(20):e2206957. doi:10.1002/advs.202206957
162. 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. doi:10.1016/j.apsb.2024.03.014
163. Kiran GS, Ninawe AS, Lipton AN, et al. Rhamnolipid biosurfactants: evolutionary implications, applications and future prospects from untapped marine resource. *Crit Rev Biotechnol*. 2016;36(3):399–415. doi:10.3109/07388551.2014.979758
164. Shen Y, Zou Y, Chen X, et al. Antibacterial self-assembled nanodrugs composed of berberine derivatives and rhamnolipids against *Helicobacter pylori*. *J Control Release*. 2020;328:575–586. doi:10.1016/j.jconrel.2020.09.025
165. Cai J, Huang H, Song W, et al. Preparation and evaluation of lipid polymer nanoparticles for eradicating *H. pylori* biofilm and impairing antibacterial resistance in vitro. *Int J Pharm*. 2015;495(2):728–737. doi:10.1016/j.ijpharm.2015.09.055
166. Qiao Y, Han M, Fu H, et al. Mucus-penetrating nanoassembly as potential oral phototherapeutic formulation against multi-drug resistant *Helicobacter pylori* infection. *Small*. 2024;20(20):e2306909. doi:10.1002/smll.202306909
167. Barreto JA, O'Malley W, Kubeil M, et al. Nanomaterials: applications in cancer imaging and therapy. *Adv Mater*. 2011;23(12):H18–40. doi:10.1002/adma.201100140
168. Nazir F, Tabish TA, Tariq F, et al. Stimuli-sensitive drug delivery systems for site-specific antibiotic release. *Drug Discov Today*. 2022;27(6):1698–1705. doi:10.1016/j.drudis.2022.02.014
169. Ejaz S, Ejaz S, Shahid R, et al. Chitosan-curcumin complexation to develop functionalized nanosystems with enhanced antimicrobial activity against hetero-resistant gastric pathogen. *Int J Biol Macromol*. 2022;204:540–554. doi:10.1016/j.ijbiomac.2022.02.039
170. Mosallam FM, Bendary MM, Elshimy R, et al. Curcumin clarithromycin nano-form a promising agent to fight *Helicobacter pylori* infections. *World J Microbiol Biotechnol*. 2023;39(12):324. doi:10.1007/s11274-023-03745-7
171. Wu Y, Geng J, Cheng X, et al. Cosmetic-derived mannoseylerythritol 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. doi:10.1021/acsomega.2c02953
172. Huang Y, Li R, Dai Y, et al. Rhamnolipid-assisted black phosphorus nanosheets with efficient isolinderalactone loading against drug resistant *Helicobacter pylori*. *Mater Des*. 2022;216:110536. doi:10.1016/j.matdes.2022.110536
173. Qiu H, Gong L, Slezak P, et al. Magnetic-navigable silk fibroin microneedles for oral drug delivery: ensuring long-lasting *helicobacter pylori* eradication and rapid hemostasis in the stomach. *Int J Biol Macromol*. 2024;275(Pt 1):133584. doi:10.1016/j.ijbiomac.2024.133584
174. Jiang H, Li L, Li Z, et al. Metal-based nanoparticles in antibacterial application in biomedical field: current development and potential mechanisms. *Biomed Microdevices*. 2024;26(1):12. doi:10.1007/s10544-023-00686-8
175. Drulis-Kawa Z, Dorotkiewicz-Jach A. Liposomes as delivery systems for antibiotics. *Int J Pharm*. 2010;387(1–2):187–198. doi:10.1016/j.ijpharm.2009.11.033
176. Dymek M, Sikora E. Liposomes as biocompatible and smart delivery systems - the current state. *Adv Colloid Interface Sci*. 2022;309:102757. doi:10.1016/j.cis.2022.102757
177. Andra V, Pammi SVN, Bhatraju L, et al. A comprehensive review on novel liposomal methodologies, commercial formulations, clinical trials and patents. *Bionanoscience*. 2022;12(1):274–291. doi:10.1007/s12668-022-00941-x
178. Banerjee A, Chowdhury P, Bauri K, et al. Inhibition and eradication of bacterial biofilm using polymeric materials. *Biomater Sci*. 2022;11(1):11–36. doi:10.1039/d2bm01276f

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