Review

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Nanobiomaterials Based Sonodynamic Therapy for Treament of *Helicobacter pylori* Infections: A Review

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Abstract

Helicobacter pylori (HP) is a helical-shaped bacterium that inhabits the human stomach and is associated with various pathologies, including gastritis, gastric ulcers, and gastric cancer. HP infection is the largest contributor to gastric cancer, and approximately 90% of non-cardia gastric cancers are related to HP infection. As HP gastritis is an infectious disease, eradicating HP is an effective measure to prevent gastric cancer. Traditional triple therapy has demonstrated limited efficacy due to the emergence of antibiotic resistance and disruption of the intestinal microbiota. Sonodynamic therapy is an innovative nonantibiotic approach that utilizes a sonosensitizer to generate reactive oxygen species in response to ultrasound, effectively targeting pathogenic microorganisms; recent advancements have highlighted its potential for the treatment of HP infection. This article reviews recent developments in ultrasound-assisted biomaterials designed to combat HP while simultaneously preserving intestinal microecology. Furthermore, it discusses the integrated mechanisms underlying both the anti-HP effects and the maintenance of intestinal microecology. These strategies provide novel insights into overcoming the limitations associated with traditional antibiotic therapies and establish a foundation for future clinical applications.

Keywords: *Helicobacter pylori* (HP); ultrasonic; sonodynamic therapy; biomaterials; intestinal microbiota

Introduction

Helicobacter pylori (HP), a spiral-shaped, microaerophilic Gram-negative bacterium, infects approximately half of the global population, primarily residing in the human stomach where it can trigger gastritis, gastric ulcers, and gastric cancer, among other gastric diseases [1–4]. Moreover, HP has been implicated in the development of extraintestinal conditions, including idiopathic thrombocytopenic purpura, unexplained iron deficiency anemia, and vitamin B12 deficiency [5, 6]. HP was classified as a Group 1 carcinogen by the World Health Organization in 1994 [7]. Subsequent international

authoritative consensuses, including the 2015 Kyoto Global Consensus on HP Gastritis and the 2017 Maastricht V Consensus, have emphasized that HP gastritis is an infectious disease and that HP should be eradicated to prevent gastric cancer. These consensus statements recommend that all individuals infected with HP undergo eradication therapy, unless there are compelling contraindications [8, 9]. Indeed, a systematic review and meta-analysis have shown that eradicating HP can significantly lower the incidence of gastric cancer [10]. In the latest large-scale prospective study (involving 180 284 subjects) published in *Nature Medicine* in 2024, a team from Peking University confirmed that eradicating HP can reduce the risk of gastric cancer by nearly 20%. The study also showed that eradicating HP could prevent more than 85 000 cases of stomach cancer annually in China [11], which was further substantiated by a nationwide, multi-center, long-term follow-up study [12]. Therefore, eradicating HP to prevent gastric cancer and associated diseases is crucial for safeguarding public health.

Following its discovery in 1982 [13], HP infection has posed a significant global medical challenge. Standard treatments involve triple therapy, which pairs the proton pump inhibitor omeprazole with two antibiotics (amoxicillin and clarithromycin) or quadruple therapy, which includes bismuth in addition to the triple therapy regimen [9, 14]. The extended duration of these treatments, typically ranging from 7 to 14 days, along with the need for concurrent administration of multiple drugs, results in high medical costs and low patient compliance, thereby diminishing their effectiveness [15]. Moreover, overuse of antibiotics has led to a growing global resistance rate in HP. HP's long-term co-evolution with humans has enabled it to invade gastric epithelial cells, creating intracellular sanctuaries that shield it from both cellular and antibiotic elimination, and to form bacterial biofilms, which contribute to its drug resistance [16,17]. A meta-analysis reported increased resistance rates to clarithromycin and levofloxacin, reaching 21% and 27%, respectively, in the Asia-Pacific region between 2011 and 2015 [14]. Escalating drug resistance complicates the reliance on antibiotics for treating HP infections. Furthermore, antibiotics can disrupt the gastrointestinal microbiota, precipitating additional diseases [18].

In response to the pressing clinical issues posed by HP infection, Zhaoshen Li and Yiqi Du, leading digestive disease experts, have spearheaded our team—comprising the National Clinical Medical Research Center for Digestive Diseases and the Department of Gastroenterology and Clinical Research Center at the First Affiliated Hospital of Naval Medical University—in conducting several high-level studies [19, 20]. We completed a large-scale, nationwide, family-based epidemiological survey of HP infection, which revealed an average individual infection rate of 40.66% and an overall family infection rate of 71.21% in China [19]. Additionally, we established the inaugural consensus on household prevention and control of HP [20]. Our research on HP prevention and control has garnered international acclaim, having been featured as a significant medical advancement in the Health and Environment China 2023 report, and was endorsed by Academician Marshall, the discoverer of HP and Nobel Laureate in Medicine. In a review published in 2023 in *Gut*, Academician Marshall highlighted that family-based screening and eradication of HP in China are a viable and promising strategy [21]. While eradicating HP is pivotal for safeguarding public health and alleviating its medical burden, there is an imperative need to devise innovative therapeutic strategies aimed to not only eradicate HP but also preserve the intestinal microbiome.

In recent years, biomaterials have been included in antibacterial strategies for clinical translation as a potent alternative to traditional antibiotic regimens (Fig. 1) [22, 23]. Biomaterials harbor distinct advantages in antimicrobial activity owing to their customizable physical and chemical properties [24]. Precise control over the shape, size, and surface chemistry of these materials endows them with inherent antibacterial capabilities [25]. Furthermore, biomaterials serve as effective drug delivery vectors, enabling the sustained release of therapeutics and the precise targeting of bacterial pathogens, thereby mitigating the emergence of drug-resistant strains [26, 27]. Additionally, by integrating biomaterials with various substances, multifunctional antibacterial platforms can be engineered, boasting a range of benefits such as mucosal repair, intestinal flora preservation, and immune regulation [28, 29]. Among these emerging treatment technologies, sonodynamic

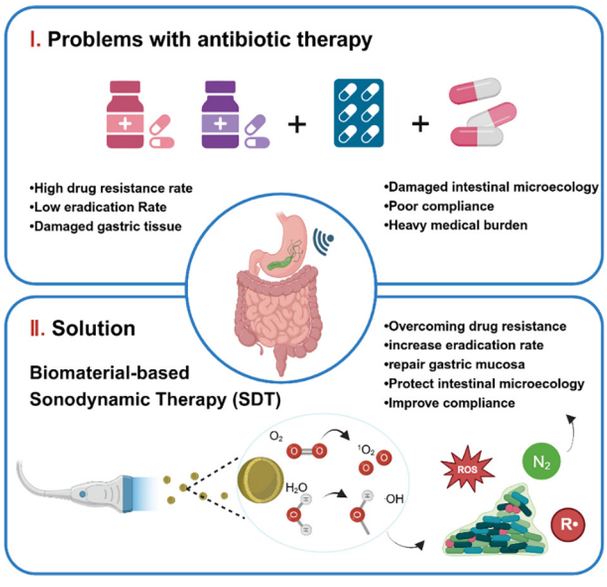

Fig. 1 SDT anti-HP solves the problem of drug resistance and intestinal microecological damage. Created with BioRender.com.

therapy (SDT) stands out for its noninvasive nature, profound tissue penetration, proven safety, and absence of drug resistance [30]. By promoting the generation of reactive oxygen species (ROS) and leveraging the mechanical effects of ultrasonic cavitation, SDT exerts antimicrobial effects under ultrasonic stimulation [31]. This approach has demonstrated substantial potential in combating HP infections, effectively eradicating the pathogen while concurrently safeguarding the intestinal microbiome and preserving beneficial bacteria [32].

Notably, our team has published a paper related to the eradication of HP in *Acta Pharmaceutica Sinica B* [33]. In addition, we conducted a systematic review on the eradication of HP via nanomaterials, and we presented our perspectives in journals such as *Gut Microbes* [34] and *International Journal of Nanomedicine* [35]. In this review, we provide a comprehensive overview of the current landscape and the challenges in HP treatment. We delve into the application of SDT-assisted biomaterials in the management of HP infections, encompassing the development of diverse acoustic sensitizers, the antibacterial mechanisms of SDT, and strategies for preserving the intestinal microbiota. Moreover, we examine the integrated mechanisms of SDT in treating HP infections and inform future research directions and clinical applications for replacing conventional antibiotic therapies.

HP Treatment Status and Problems

Status quo of treatment based on antibiotics

HP is the primary etiological agent responsible for a spectrum of gastric and extra-gastric diseases, including chronic gastritis, duodenal ulcer, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer [36]. Since its initial identification in 1982, HP infection has garnered attention among researchers worldwide [37]. Historically, the standard of care involved triple therapy, comprising proton pump inhibitors (PPIs), amoxicillin, and clarithromycin, which was widely recommended for HP eradication. However, escalating rates of antibiotic resistance have significantly decreased the efficacy of this regimen [38]. In response, Fifth Chinese National Consensus Report on HP infection management has endorsed bismuth-containing quadruple therapy (PPI + bismuth + two antibiotics) as the preferred empirical treatment for HP eradication, with a recommended duration of 10 to 14 days [39]. The Maastricht VI/Florence Consensus report also affirmed the use of bismuth-containing quadruple and triple therapies as first-line treatments for HP infection [1]. Despite these advancements, no treatment has been able to completely eradicate HP as antibiotic therapies continue to face a myriad of challenges.

Problems faced by antibiotic therapy

Antibiotic resistance

Eradication of HP has been proven to significantly diminish the risk of gastric cancer [40]. However, the efficacy of HP eradication therapy is declining due to the rise in antimicrobial resistance and, to a lesser extent, the variable efficacy of acid-suppressing drugs [41]. In clinical practice, antibiotic therapy remains the cornerstone for HP eradication, with commonly utilized antibiotics including clarithromycin, metronidazole, amoxicillin, levofloxacin, furazolidone, and tetracycline, among others [42]. The irrational use of antibiotics has contributed to an increasing resistance rate in HP, with metronidazole, plazomicin, and levofloxacin showing particularly high levels of resistance. In contrast, amoxicillin exhibits a lower resistance rate, yet it too is trending upward annually [42].

Research into the mechanisms of drug resistance has intensified in recent years. Clarithromycin exerts its bactericidal effects by binding to the 50S subunit of bacterial ribosomes, thereby disrupting protein synthesis. As early as 1996, studies have attributed point mutations in the peptidyl transferase region (V domain) of the 23S rRNA within the 50S ribosomal subunit to HP's clarithromycin resistance [43]. Furthermore, mutations in the hp1314 (rpl22) gene, encoding ribosomal protein L22, and the hp1048 (infB) gene, encoding translation initiation factor IF-2, have been implicated in clarithromycin resistance and are known to exhibit synergistic effects with 23S rRNA mutations [44, 45]. Studies have also associated mutations in the *rdxA* (oxygen-insensitive NADPH nitro reductase), *frxA* (NADPH flavin REDOX reductase), and *fdxB* (ferredoxin analogs) genes with varying levels of metronidazole resistance. Notably, a single mutation in *fdxB* results in low or no resistance to metronidazole in HP, whereas mutations in *rdxA* and *frxA* are linked to high levels of resistance [46]. The resistance of HP to levofloxacin is predominantly associated with Asn-87 and/or Asp-91 mutations in the quinolone resistance-determining region of *gyrA* [47].

Additionally, HP's ability to form biofilms both *in vivo* and *in vitro* can augment its resistance to clarithromycin, amoxicillin, and metronidazole *in vitro* [48]. Extracellular polymers (EPS), located in the biofilm's outermost layer, play a crucial role in HP biofilm resistance by shielding the enclosed cells from direct interaction with human immune cells and reducing the permeability of antibiotics [49]. Efflux pump activity and virulence factors may also contribute to drug resistance. In summary, antibiotic resistance in HP poses a global challenge that requires urgent attention, necessitating comprehensive investigations into resistance mechanisms, judicious antibiotic stewardship, and innovative therapeutic strategies.

Damaged intestinal microecology

The human gut microbiome is a critical microbial organ during both health and disease states [50]. Commencing from birth, the gut microbiota adheres to the gut epithelium and neighboring organs, where it participates in the digestion and transformation of nutrients, establishes colonization resistance against invasive pathogens, and modulates the immune system [51]. Disruptions in gut microbiota can severely compromise human health. During the course of HP treatment, antibiotics not only target and eliminate HP but also disrupt the indigenous gut microbial community, triggering an imbalance in the intestinal microbiota [52]. Typically, antibiotic exposure, irrespective of the drug class, induces significant alterations in the microbial community structure, species composition, and metabolic potential. Fundamental research in healthy adults generally documents a precipitous drop in alpha diversity shortly after treatment initiation [53] and an incomplete restoration of the microbiome even six months post-treatment, as assessed by beta diversity comparisons between pre- and post-treatment states [54–56]. Currently, bismuth quadruple therapy (BQT) is advocated as the first-line treatment for HP eradication. However, increasing resistance to clarithromycin, metronidazole, and levofloxacin has led to a gradual decline in BQT's efficacy. Furthermore, eradication regimens containing clarithromycin have been shown to elevate levels of the macrolide resistance gene (*ermB*) in the gut microbiome [57, 58]. Prior research indicates that although BQT can induce intestinal microbiota imbalance, this disruption is typically followed by a gradual recovery within one year [59, 60]. Disruption of the intestinal microbiota is a significant concern in antibiotic-based HP eradication therapies. Consequently, there is an imperative need to develop novel therapeutic strategies that not only eliminate HP but also safeguard the integrity of the intestinal microbiota.

Poor compliance

Achieving a high level of adherence to drug therapies is essential for successful treatment outcomes, as suboptimal adherence is a significant predictor of treatment failure [61, 62], yet this issue is often underrecognized [63]. Several factors contribute to poor patient compliance, including the complexity and multitude of medications, the extended treatment duration, and the presence of side effects. Standard treatment regimens for HP eradication typically involve triple therapy, which includes a proton pump inhibitor (PPI), amoxicillin (1 g twice daily), and clarithromycin (500 mg twice daily), or a quadruple therapy comprising a PPI, bismuth subsalicylate, and two antibiotics (metronidazole and tetracycline) for 14 days in patients with identified risks for macrolide resistance. Patients are often required to take 6612

tablets daily, which, given the variety of drugs, presents a substantial risk for confusion and nonadherence [64]. The complexity of these regimens, characterized by multiple daily doses and serious side effects such as abdominal pain, headache, nausea, vomiting, and diarrhea, frequently leads to treatment abandonment or incomplete compliance [65, 66]. Indeed, the failure rate of HP eradication is 4.26 times higher in patients who discontinue treatment compared with those who maintain adherence [67]. Therefore, enhancing patient compliance and emphasizing health education during treatment are essential.

SDT Anti-HP Protects Intestinal Microecology

SDT, which has recently emerged as a promising treatment modality, relies on three fundamental components: low-frequency and low-intensity ultrasound, an acoustic sensitizer, and molecular oxygen. Imperative for the therapeutic efficacy of SDT is the acoustic sensitizer, which is typically derived from photosensitizers but can suffer from drawbacks such as poor solubility, low stability, inadequate targeting, and a propensity for aggregation [68] that restrict their *in vivo* applications. The integration of nanotechnology has been instrumental in reducing the threshold for ultrasonic cavitation, thereby enhancing the generation of hydroxyl radicals (•OH) and bolstering the antibacterial properties of SDT [69]. Compared to their traditional counterparts, nano-ultrasonic sensitizers more efficiently accumulate in infected regions thanks to enhanced hydrophilicity and prolonged circulation times within the body. These nanoscale biomaterials facilitate cellular uptake of nanosensitizers and promote deep tissue penetration, while also improving aqueous solubility and biocompatibility without altering the sensitizer's inherent physical and chemical characteristics [70]. Below, we detail the acoustic sensitizers employed in SDT for treating HP infection (Table 1).

Organic sound sensitizer

Indole

Indocyanine green (ICG) is a thiacarbocyanine dye with a characteristic near-infrared (NIR) absorption peak that is extensively utilized in fluorescence imaging [79]. Approved by the US Food and Drug Administration (FDA) for clinical trials in 1956, ICG is renowned for its biosafety [80] and can generate singlet oxygen ($^1O_2$) under ultrasound (US) irradiation, highlighting its potential as an acoustic sensitizer [81,82]. Nomikou et al. reported that ICG exhibits excellent biocompatibility, causing minimal cell death even at high concentrations. However, significantly reduced cell viability was observed when ICG-treated cells were subjected to US irradiation [81]. The reaction product of

Table 1 Representative acoustic sensitizer in SDT.

Sound sensitizer	Ultrasound parameters	Antibacterial effects of SDT	Ref.
Organic sound sensitizer			
CNPS-ICG	1 MHz, 1.56 W/cm², 1 min	Removed periopathogen biofilm on the surface of the titanium dental implant under diode laser irradiation + US	[71]
HpAb-LiP-ICG	1 MHz, 1.5 W/cm², 10 min	Activated Ab-LiP-ICG produces singlet oxygen ($^1O_2$), which can effectively damage the cell membrane and internal structure of bacteria.	[72]
HMME	1 MHz, 3 W/cm², 10 min	Treating *P. gingivalis* with ultrasound at a concentration of 40 μg/mL HMME at 3 W/cm² for 10 minutes reduced CFU (colony forming unit) by 4.7 lg ($p < 0.01$).	[73]
Fe-HMME@DHA@MPN	1 MHz, 1.5 W/cm², 70% cycle, 4 min	Ultrasonic treatment, improved biofilm activity, with a removal rate of 94.5%	[74]
Fe@UCNP-HMME	2 W/cm², 10 min	Achieved 0% of relative viability	[75]
Ver-PLGA@Lecithin	1 MHz, 0.5 W/cm², 70% cycle, 10 min	Under the action of ultrasound, VerPLGA@Lecithin nanoparticles can generate ROS and effectively kill HP	[32]
Inorganic sound sensitizer			
PtCu-PEG NPs	40 kHz, 3 W/cm², 50% cycle, 8 min	The partial oxygenation formed on the surface of the PtCu NPs endows them with good Fenton-like catalytic performance and superior GSH-depleting ability, thus enhancing reactive oxygen species generation.	[76]
PPAF	1 MHz, 1 W/cm², 50% cycle, 2 min	Penetrate the mucous layer, target HP, destroy the biofilm, and show excellent bactericidal ability.	[77]
Pd@Pt-T790	1 MHz, 0.97 W/cm², 50% cycle, 8 min	Nearly 100% of the bacteria were killed	[78]

dihydrofluorescein (DHFA) with $^1O_2$, indicative of $^1O_2$ generation by ICG under US irradiation, was observed in RIF-1 cells, generating a strong fluorescence signal. Notably, the combination of US and light resulted in up to 90% cell mortality. A mouse tumor xenotransplantation model showed that this method could effectively inhibit tumor growth by 98%. In 2022, Wang et al. pioneered the use of SDT for HP infection, developing monoclonal antibody-coupled liposomes (HpAb-LiP-ICG) loaded with ICG as photoacoustic (PA) image-guided SDT for HP infection. These liposomes demonstrated high stability and biocompatibility in acidic environments (pH 1.5), making them suitable for the oral treatment of HP-infected mice. PA imaging confirmed the accurate identification and targeting of gastric HP by HpAb-LiP-ICG. Upon targeting HP, ICG is activated by US irradiation to produce $^1O_2$, thereby destroying HP. Recently, Yin et al. developed an innovative material called ICG@FCS that is formed by covalently binding ICG to two biocompatible polysaccharides: chitosan (CS) and focus (FS). ICG@FCS demonstrated the ability to penetrate mucus layers, selectively identify and target HP, and produce ROS when stimulated by US. This antimicrobial activity not only clears bacterial biofilms, but also stimulates autophagy to eliminate intracellular bacteria. In addition, ICG@FCS helps repair the gastric mucosa and maintains the integrity of the intestinal flora, offering a multifaceted approach to combat HP infection (Fig. 2) [83].

Porphyrin

Porphyrins, with their 18-π aromatic macrocyclic carbon core structure, are known for efficiently producing photoinduced $^1O_2$. They have been approved by the US FDA for clinical use in photodynamic therapy (PDT) [84]. Hematoporphyrin monomethyl ether (HMME) is an effective acoustic sensitizer in SDT, characterized by structural stability, low dark toxicity, and high singlet oxygen

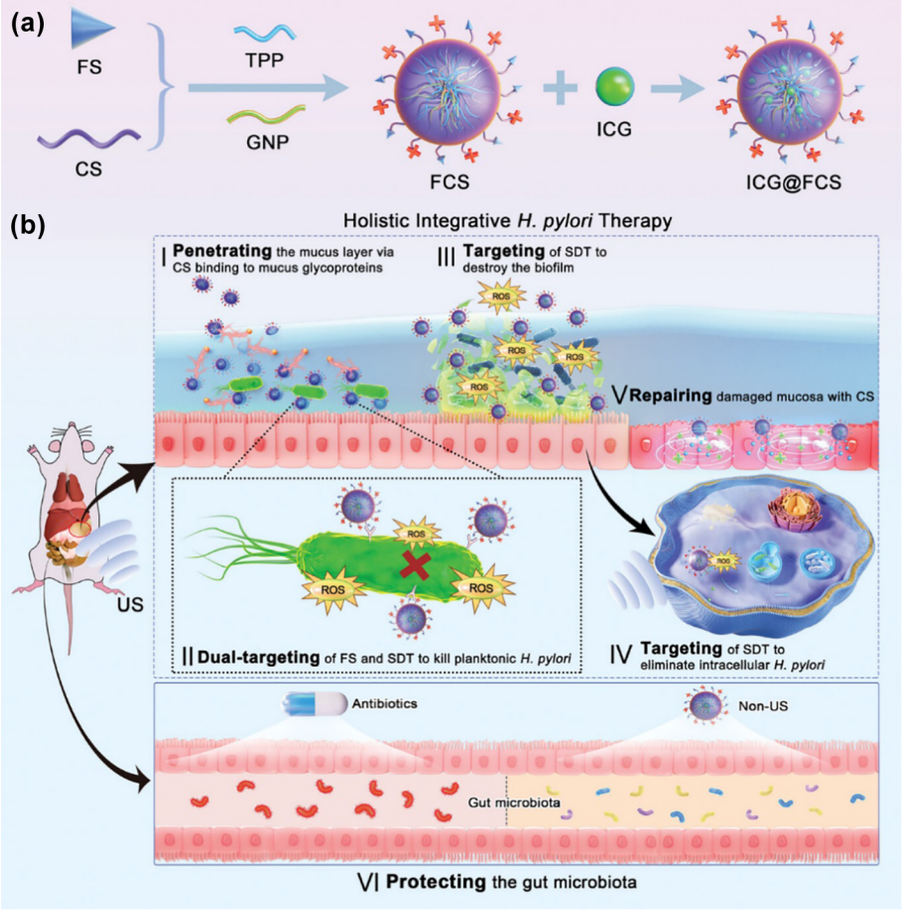

Fig. 2 Schematic of the eradication of HP via the SDT-based ICG@FCS nanoplatform. **(a)** Preparation of FCS and ICG@FCS. **(b)** ICG@FCS penetrates the mucus layer, specifically recognizes and targets HP, generates ROS under US stimulation to exert antimicrobial activity, eradicates biofilms, promotes autophagy to clear intracellular bacteria, and simultaneously repairs the gastric mucosa and protects the gut microbiota Reproduced with permission from Ref. [83] © 2024 The Authors.

production, inducing cell apoptosis through the mitochondrial pathway [85]. Ma et al. conjugated a mitochondrial targeting probe (TPP) with cholesterol (Chol) to create TPP-Chol, which assembles with HMME into liposomes through strong hydrophobic interactions. The resulting TPP-Lipo-HMME nanoparticles target mitochondria, rapidly release HMME under US irradiation, generate $^1O_2$, and induce cell death. *In vitro* experiments showed significantly lower viability of MCF-7 cells treated with TPP-Lipo-HMME compared with Lipo-HMME [86]. HMME, apart from its use in activating US to produce ROS for cancer treatment, has also been explored as a sensitizer in acoustic sonodynamic antimicrobial chemotherapy (SACT) against *Porphyromonas gingivalis*. Zhang et al. found that SACT generated ROS, effectively inhibiting the growth of *P. gingivalis in vitro* [87]. HMME-mediated SDT has shown significant efficacy in eradicating HP. Yu et al. prepared a novel metal-organic hybrid nanomaterial with pH-responsive peroxase-like activity for combined chemodynamic therapy (CDT) and SDT antibacterial action against HP [74]. The ROS nanogenerators catalyze H_2O_2 in the infected microenvironment or dehydroascorbic acid (DHA) incorporated into the nanogenerators, continuously generating ROS in an acidic environment through Fenton/Fenton-like reactions for CDT. Under US irradiation, oxygen produced by the Fenton reaction of SDT further promoted ROS generation (Fig. 3). The combination of CDT and SDT rapidly eliminates both drug-sensitive and drug-resistant HP without harming symbiotic bacteria and mammalian cells while removing biofilms. Furthermore, antipool, a benzoporphyrin derivative [88], has been identified as a potential acoustic sensitizer in SDT. Moreover, Yang et al. loaded three acoustic sensitizers — vitipofol, ICG, and chlorin (Ce6) — into lecithin double-coated PLGA nanoparticles (PLGA@Lecithin), obtaining three USS-PLGA@Lecithin nanoparticles with similar size and surface charge [32]. After comparing the effects

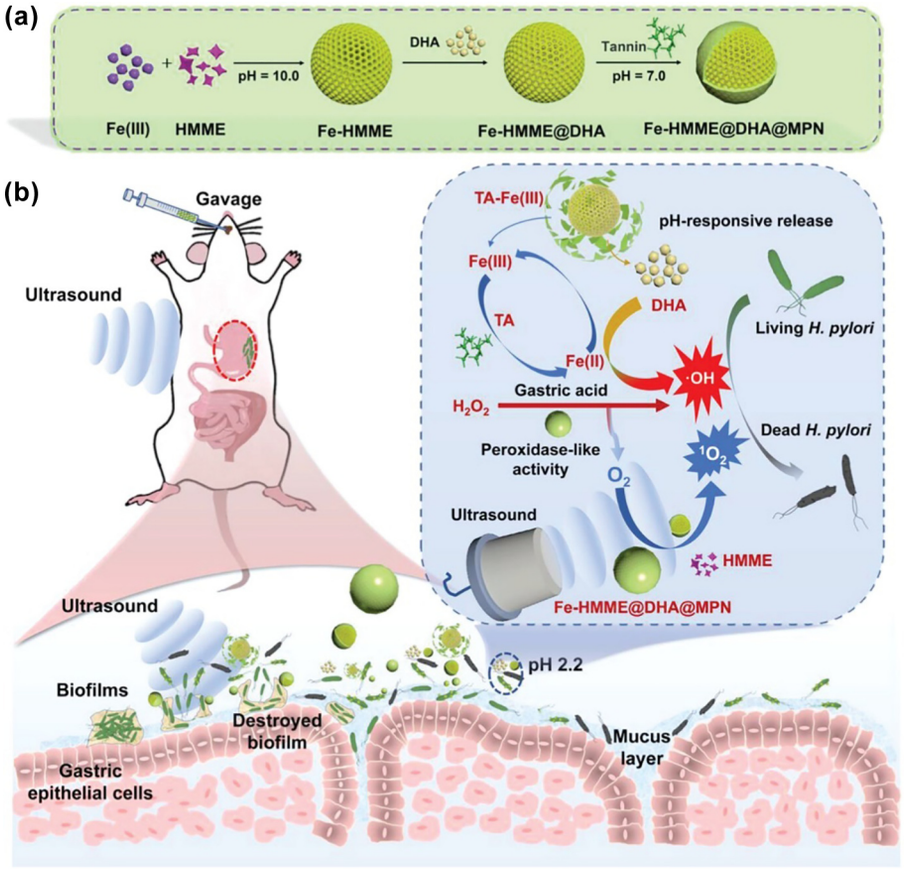

Fig. 3 Schematic of the functional mechanism of the ROS nanogenerator Fe-HMME@DHA@MPN. **(a)** Preparation of the nanogenerator. **(b)** The therapeutic mechanism of the nanogenerator for the treatment of HP infection through the synergy of sonodynamic therapy (SDT) and chemodynamic therapy (CDT) under ultrasound treatment. HMME: sonosensitizer hematoporphyrin monomethyl ether; DHA: anti-malarial drug dihydroartemisinin; TA: polyphenol tannic acid. Reproduced with permission from Ref. [74] © 2023 The Authors.

of these nanoparticles on ROS generation and HP, Ce6-PLGA@Lecithin and Ver-PLGA@Lecithin were found to be relatively efficient, whereas ICG-PLGA@Lecithin was least efficient. Considering that verteporfin has been approved for clinical use by the FDA in the United States and Ce6 has not, Yang et al. selected Ver-PLGA@Lecithin as a nanosonic sensitizer for SDT.

Inorganic acoustic sensitizer

Inorganic acoustic sensitizers enhance the efficacy of SDT through their superior chemical and physiological properties and versatility. Cheng et al. developed pegylated platinum-copper nanoparticles (PtCu-PEG NPs) as a novel acoustic sensitizing agent for effective bacterial elimination [76]. The PtCu-PEG NPs demonstrated excellent broadband bactericidal capability through SDT performance, Fenton-like catalysis, and glutathione depletion activity. In addition, PtCu-PEG NPs promoted angiogenesis by upregulating hypoxia-inducing factor-1 (HIF-1α), platelet endothelial cell adhesion molecule 31 (CD31), and increased M1 and M2 macrophage levels for bacterial clearance. Under US irradiation, the PtCu-PEG nanoplatform-mediated SDT and glutathione-enhanced chemodynamic therapy-mediated combination therapy successfully eliminated osteomyelitis. Furthermore, $PtCu_3$, an efficient ROS generator that is highly hydrophobic, was incorporated into the sonodynamic nanocomposite $PtCu_3$-PDA@AIPH@Fucosan (PPAF), enhancing its hydrophilicity through PDA modification. PPAF penetrates the mucus layers, targets HP, destroys biofilms, and exhibits excellent bactericidal capabilities (Fig. 4) [77]. Due to their tunable structure, special physicochemical properties, high chemical stability, and excellent ROS generation under ultrasonic irradiation with lower phototoxicity than organic sensitizers, titanium-based nanoacoustic sensitizers have been designed and applied to antimicrobial and antibiofilm SDT-based therapies [89]. For instance, Zhu et al. constructed a new slime mold gel comprised of $Ti_2C(OH)_2$ nanosheets dotted with amorphous TiOx nanofibers, which effectively inhibited bacterial invasion by binding to bacteria. The nanocomposite induced significantly more ROS generation than TiO_2 NPs, and $Ti_2C(OH)_2$; catalase-like activity provided sufficient oxygen at the infection site to promote bacterial killing by SDT, suggesting its potential as an SDT antibacterial agent for killing bacteria and promoting wound healing [90]. Therefore, we hypothesize that titanium-based nanoacoustic sensitizers could also be effective in eradicating HP, warranting further exploration.

SDT-assisted biomaterials for HP resistance

The bacterial cell envelope possesses three key signal recognition sites: phospholipids, proteins, and nucleic

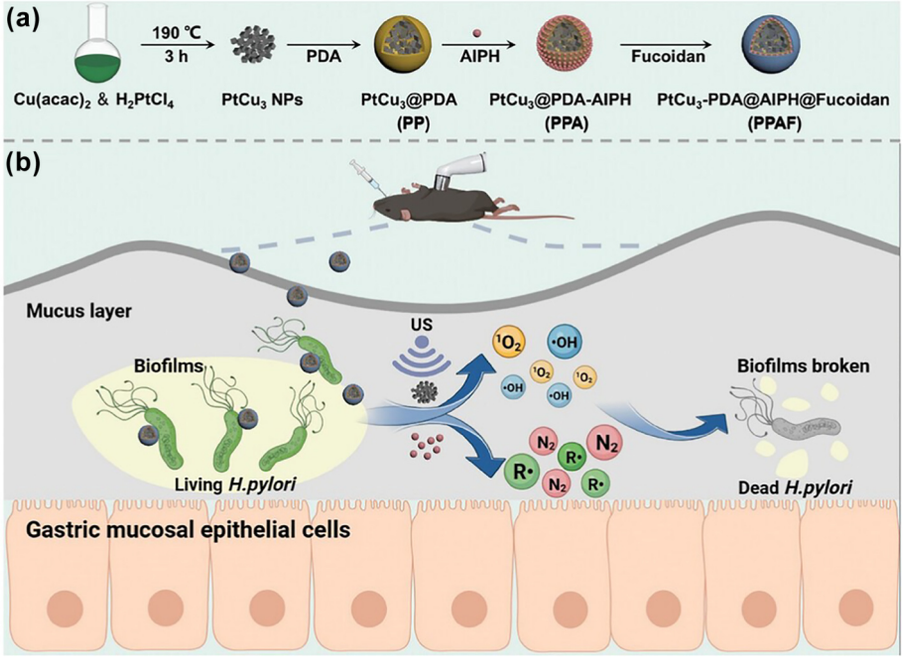

Fig. 4 Schematic of the functional mechanism of PPAF. **(a)** Preparation of PPAF. **(b)** Therapeutic mechanism of SDT in the treatment of *H. pylori* infection under ultrasound therapy. Reproduced with permission from Ref. [77] © 2024 Wile-VCH.

acids on the cell membrane [91]. Ultrasonic stimulation can induce morphological and functional changes in microbial cells by interfering with these sites. Current research indicates that the primary mechanism of SDT against HP involves ROS generation, with additional contributions from the physical damage caused by US irradiation to bacterial cells [92]. In the subsequent sections, we discuss potential antimicrobial mechanisms during US stimulation, including ROS production mediated by sonar sensitizers and auxiliary antimicrobial mechanisms during US irradiation.

SDT resistance to planktonic HP-resistant bacteria

Numerous experiments have demonstrated that US can stimulate ROS production through acoustic sensitizers [93, 94]. When acoustic sensitizers or endogenous molecules are exposed to US, they transition from the ground state to the excited state. The subsequent release of energy upon returning to the ground state can generate ROS, which damage bacteria and eventually trigger a cascade of events leading to bacterial apoptosis [95, 96]. To leverage this mechanism, Liu et al. developed a novel nanoparticle, Ver-PLGA@Lecithin, composed of polylactic acid-glycolic acid (PLGA) nanoparticles coated with lecithin double layers and preloaded with the sensitizer Vitipofol (Ver). This nanoparticle binds to HP cell surface proteins and forms a complex in the acidic gastric environment. Activated by nondestructive ultrasonic waves, the nanoparticles produce ROS, which are then directed noninvasively to HP. ROS production leads to cell membrane damage and oxidative stress in bacteria, achieving efficient bacterial inactivation [32]. Application of the Ver-PLGA@Lecithin + US treatment group (0.5 W/cm² for 10 min) in a female mouse model infected with HP was as effective as the standard triple therapy, significantly alleviating HP infection in the stomach. Notably, the mean gastric HP burden in the Ver-PLGA@Lecithin + US group was comparable to that of the triple therapy group, yet approximately 50 times lower than that of the untreated control group. However, the efficiency of traditional acoustic sensitizers in ROS production is limited by their electronic transition capacity and oxygen content [97]. Given HP's microaerophilic environment, alternative methods to alleviate hypoxia, such as in situ oxygen generation or carrier oxygen delivery, have been explored [98]. Yu et al. prepared a pH-responsive reactive oxygen species nanogenerator (Fe-HMME@DHA@MPN) that generates more singlet oxygen under US than the sensitizer HMME alone. The Fenton/Fenton-like reaction in the sonodynamic process, driven by Fe(II) in the gastric fluid and H_2O_2 in the infected microenvironment, enhances the generation of ROS. Nanoparticles adhere to HP, forming aggregates that reduce the propagation distance of ROS and thereby their lifespan to elicit antibacterial effects. The synergistic application of SDT and CDT has demonstrated exceptionally high eradication efficiency against HP. Specifically, under conditions mimicking the gastric fluid environment, Fe-HMME@DHA@MPN nanoparticles were able to completely eradicate all HP present at a concentration of 40 μg/mL through ultrasonic treatment. Together, SDT and CDT eradicated 100% of HP under the experimental conditions. A series of *in vitro* experiments was also designed to test the efficacy of the nanoparticles against a variety of antibiotic-resistant bacteria. The results of these experiments are encouraging, with the nanoparticles showing significant bactericidal activity against resistant strains (Fig. 5) [74]. However, these methods are complex and have limited *in vivo* effectiveness. Therefore, exploring alternative ways to generate toxic free radicals independent of oxygen is critical in the treatment of HP infection.

Fan et al. synthesized a PPAF nanocomposite based on SDT and composed of $PtCu_3$ coated with polydopamine (PDA) as a carrier, loaded it with AIPH, and finally coated it with seaweed polysaccharide. PPAF exhibited excellent acoustokinetic properties, producing a large amount of ROS by generating oxidative stress when HP was stimulated by US. The ROS spread into the bacteria's interior, resulting in elevated ROS levels and promoting bacterial death. The mechanical action of US also caused bacterial damage, with more severe surface shrinkage in the PPAF + US treated group compared with the non-US-treated control group. PPAF accumulation around bacteria caused membrane penetration, protein and nucleic acid leakage, and ultimately bacterial death [77]. These strategies indicate that SDT-assisted biomaterials effectively target floating HP. In a mouse model addressing HP infection, the group subjected to treatment with PPAF + US exhibited a noteworthy reduction in bacterial load, exceeding tenfold

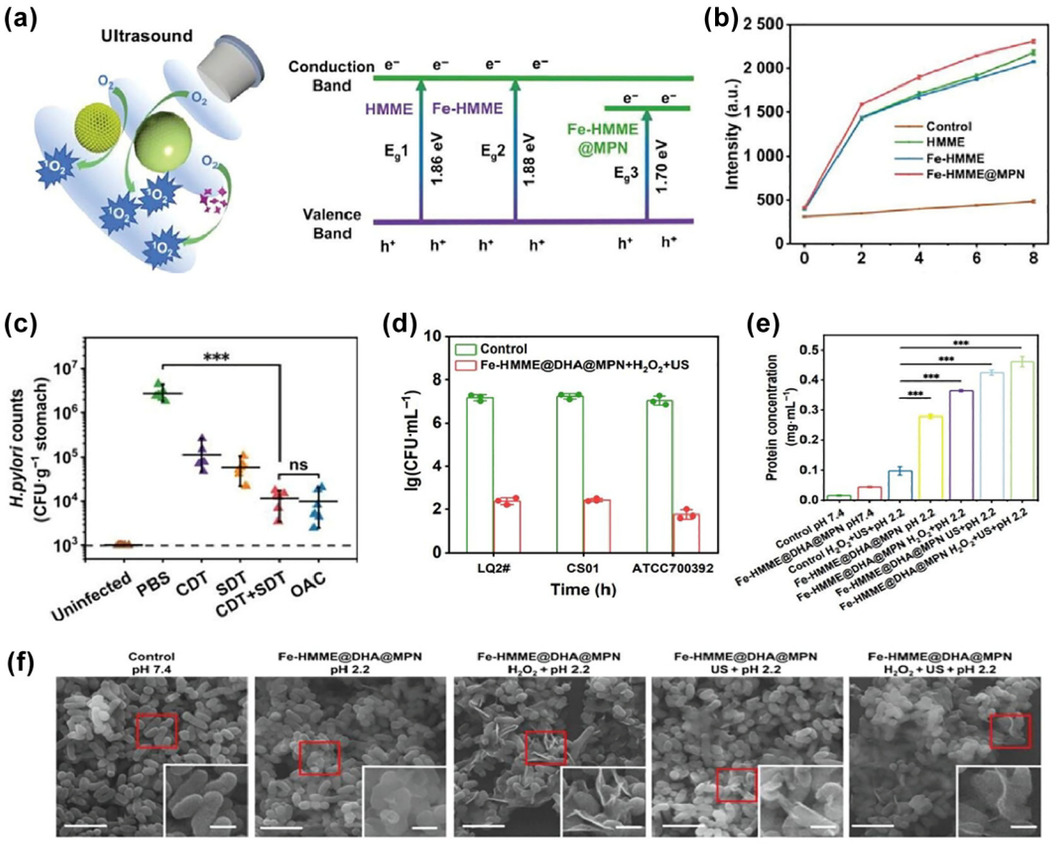

Fig. 5 Sonodynamic therapy against HP-resistant bacteria. **(a)** Schematic of the sonodynamic activity and energy bandgaps of HMME, Fe-HMME, and Fe-HMME@MPN measured by UV-Vis diffuse reflectance spectra. **(b)** Fluorescence spectra of the molecular probe singlet oxygen sensor green (SOSG) solution incubated with various samples (ultrasonication treatment time: 0–8 min). Fe-HMME@MPN generated the highest amount of $^1O_2$ (n = 3, data are presented as mean ± SD). **(c)** Quantitative results of bacterial colonies in the stomach of HP-infected mice with different treatments (PBS: phosphate-buffered saline, CDT: 30 mg/kg Fe-HMME@DHA@MPN, SDT: 16 mg/kg of Fe-HMME + US, CDT+SDT: 30 mg/kg Fe-HMME@DHA@MPN + US, OAC: combination of 400 μmol/kg omeprazole, 28.5 mg/kg amoxicillin and 14.3 mg/kg clarithromycin). Dotted line represents the limit of detection (n = 6, and ns indicates non-significance). **(d)** Colony counts of the other three HP strains LQ2#, CS01, and ATCC 700392 (initial count: 1 × 10^7 CFU/mL) after incubation with Fe-HMME@DHA@MPN (40 μg/mL) for 20 min with US treatment for 4 min in the simulated gastric fluid (pH 2.2). (n = 3, data are presented as mean ± SD). **(e)** HP protein leakage measured by BCA assay. HP (1 × 10^7 CFU/mL) was treated with ROS nanogenerators Fe-HMME@DHA@MPN (40 μg/mL) under various treatment conditions (n = 3, data are shown as means ± SD. *p < 0.05, **p < 0.01, ***p < 0.001). **(f)** SEM images of HP treated with Fe-HMME@DHA@MPN (40 μg/mL) for 20 min under various conditions, and the insets are the magnified images of the selected areas. Scale bar: 3 μm (inset: 0.5 μm). US represents ultrasonication (US treatment time: 4 min). Reproduced with permission from Ref. [74] © 2023 The Authors.

compared with the group treated with US. Although the efficacy of this combination was marginally less than that of the standard triple antibiotic regimen (OAC group), its antibacterial impact was evident.

SDT clears the HP biofilm

Biofilms are three-dimensional multicellular communities of bacteria embedded in highly resistant, self-secreted EPS [99]. After HP colonizes the gastric mucosa, it secretes proteins, polysaccharides, extracellular DNA (eDNA), and other molecules to form EPS, which are coated by bacteria and adhere to each other to form biofilms [100]. Biofilm-forming colonies are highly resistant to harsh external environments, including antibiotic exposure [48]. Generally, biofilm-forming bacteria exhibit 10–1000-fold increased resistance to antibiotics [101]. Therefore, HP biofilm formation is likely a major cause of long-term chronic infections, multiple drug resistance, and treatment failure. To eliminate HP biofilms, Yu et al. prepared a pH-responsive ROS nanogenerator (Fe-HMME@DHA@MPN) And showed improved biofilm activity after US, with a removal rate of 94.5% that can attribute to interactions between the generated ROS and the physical damage caused by US. The ROS produced through chemical and acoustic dynamics pass through the biofilm, decomposing it with ultrasonic waves to

kill bacteria deep within the biofilm (Fig. 6) [74]. Additionally, Fan et al. synthesized a PPAF nanocomposite material-assisted SDT, which not only killed floating HP through ultrasonic mechanical action but also removed bacterial biofilm. Biofilm content was further reduced when PPAF was prepared with the sonodynamic nano. This may be due to the N_2 produced by AIPH under ultrasonic conditions, which enhances the ultrasonic cavitation effect and more thoroughly breaks down biofilm. The highly dispersed biofilm allows PPAF to come into closer contact with more bacteria, improving the eradication rate of HP [77].

Mechanism of intestinal microecological protection by SDT

The gut microbiome is a key factor in regulating host health; imbalances in the gut flora can compromise human health [102]. Current treatments for HP infection often involve triple therapy with antibiotics, whose frequent and excessive use leads to intestinal flora disorders and can even cause inflammatory bowel disease and gastrointestinal cancer by hijacking host metabolism [103]. Therefore, Liu et al. developed a novel nanoparticle, Ver-PLGA@Lecithin, to address combat HP in a mouse model. Forty-eight hours after treatment, fecal samples were collected and subjected to 16S rRNA gene sequencing to comprehensively characterize the gut microbiota. The study showed that Ver-PLGA@Lecithin can effectively eradicate HP without significantly perturbing the gut microbiota, and it was as effective as standard triple therapy in reducing gastric HP infections. Importantly, SDT in the did not harm on the gut microbiota compared with the healthy group, with the predominant effect being upregulation of *Lactobacillus*, a bacterium widely recognized for its presence in yogurt products and probiotics [32]. Additionally, pH-responsive reactive oxygen nanogenerators had negligible effects on gut microbiota homeostasis. In a neutral environment (pH 7.4), the nanoparticles and their degradation products were not toxic to the symbiotic bacteria. Quantitative polymerase chain reaction analysis of the microbiome load in the stool and gut of mice showed that OAC-based treatment significantly reduced microbiome load, whereas CDT + SDT combination therapy did not. Furthermore, the microbial composition in mouse feces was analyzed by 16S rRNA sequencing, showing that OAC treatment significantly altered the microbial community structure, while the group treated with CDT + SDT showed a similar microbial community structure as the untreated control group. The α-diversity of the microbiome was assessed using the Chao 1 index, Shannon index, and Simpson index, revealing that OAC treatment enhanced the richness and diversity of the microbiome, whereas CDT + SDT treatment had no significant effect. (Fig. 7) [74].

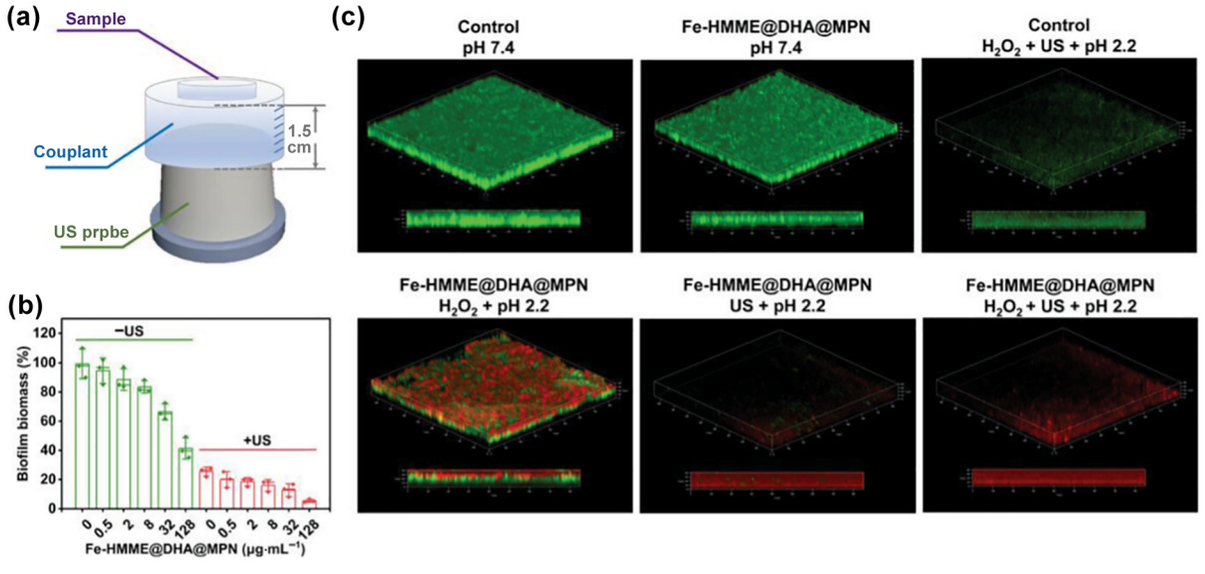

Fig. 6 Anti-biofilm activity of the catalytic ROS nanogenerator Fe-HMME@DHA@MPN against HP. **(a)** Schematic of the ultrasonic treatment. **(b)** Eradication of mature HP biofilms by treatment with Fe-HMME@DHA@MPN at various concentrations for 20 min with or without ultrasonication as shown by biofilm biomass stained with crystal violet. (n = 3, data are presented as mean ± SD). **(c)** Z-stacks of HP biofilm stained with live/dead staining kit after treatment with Fe-HMME@DHA@MPN (128 μg/mL) under various conditions for 20 min. US treatment time: 4 min. Reproduced with permission from Ref. [74] © 2023 The Authors.

Furthermore, nanocomposites prepared by Fan et al. were used to evaluate the abundance, diversity, and colony structure of intestinal microbes by 16S rRNA sequencing under US. The results showed that the PPAF + US group had little effect on the richness, diversity, and microflora structure of the intestinal microbiota of mice after treatment, with microbial diversity levels in the PPAF + US group similar to those in the uninfected group with no significant difference [77]. These results demonstrate that SDT-assisted biomaterials can not only eradicate HP but also preserve intestinal microecology during HP treatment.

Current research dedicated toward the eradication of HP through SDT-enhanced biomaterials has juxtaposed the efficacy of SDT against that of standard triple therapy to highlight the merits of the former. Our review comprehensively compiles the methodologies of SDT, delineates the ultrasonic parameters, and elucidates the antibacterial outcomes in both *in vivo* and *in vitro* settings. Furthermore, we have constructed Table 2 to concretely compare the biomaterials augmented by SDT.

Summary and Future Prospects

HP is a globally prevalent bacterium that colonizes

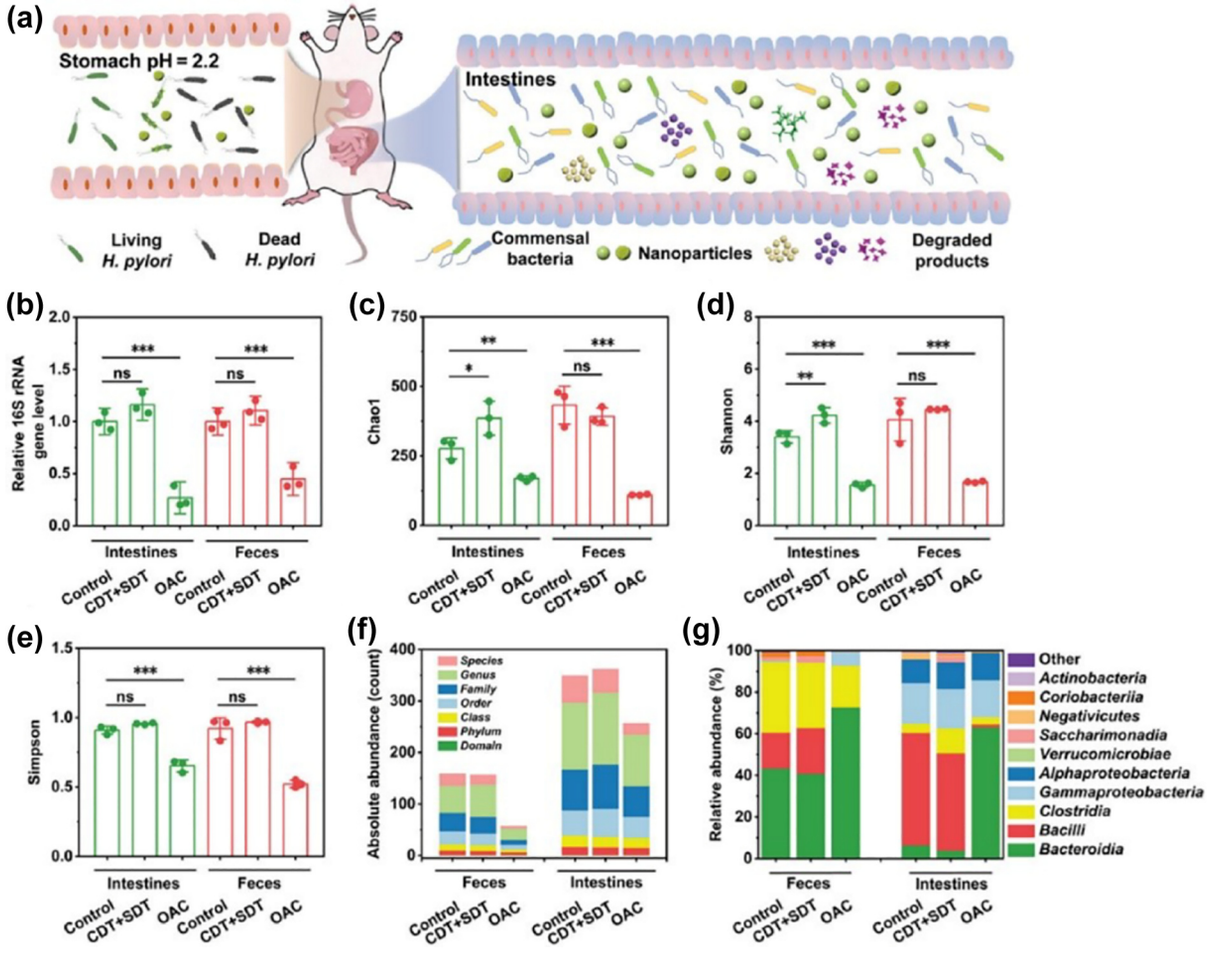

Fig. 7 Influence of CDT+SDT therapy on the homeostasis of intestinal flora in comparison with the clinically used antibiotic-based triple therapy (OAC). **(a)** Schematic diagram of the selective sterilization of *H. pylori in vivo* using the gastric acid-responsiveness of Fe-HMME@DHA@MPN. Under the acidic stomach condition, Fe-HMME@DHA@MPN catalyzed ROS production, showing significantly enhanced antibacterial efficacy. After entering the neutral intestinal environment (pH 7.4), the peroxidase-like activity of Fe-HMME@DHA@MPN was suppressed without showing a significant effect toward commensal bacteria. **(b)** Effect of Fe-HMME@DHA@MPN treatment on symbiotic bacteria in the intestines and feces of mice. Flora richness **(c)** Chao 1 diversity, **(d)** Shannon indexes, and **(e)** Simpson indexes of α-diversity in the intestines and feces of mice. **(f)** Absolute abundance of microbiota in mouse feces and intestines determined by the 16S rRNA sequencing test. **(g)** Relative abundance of microbiota structure in mouse feces determined by the 16S rRNA sequencing test. Data are presented as means ± SD (n = 3), and the significant difference was analyzed by One-way ANOVA with Tukey's post-hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 and ns representing non-significance. Reproduced with permission from Ref. [74] © 2023 The Authors.

Table 2 Comparison of the sonodynamic therapy and antibiotic therapy.

Materials	Does	Ultrasound parameters	Antibacterial rate	Antibiotic type	Antibacterial effect	Ref.
HpAb-LiP-ICG	*In vitro*: 200 μg/mL *In vivo*: 2.5 mg/kg	1 MHz, 1.5 W/cm², 10 min	*In vitro*: 99.9% *In vivo*: 99%	—	—	[72]
Fe-HMME@DHA@MPN	*In vitro*: 40 μg/mL *In vivo*: 30 mg/kg	1 MHz, 1.5 W/cm², 70% cycle, 4 min	*In vitro*: 100% *In vivo*: 99%	Triple therapy OAC	Equality	[74]
Ver-PLGA@Lecithin	*In vitro*: 100 μg/mL *In vivo*: 10 mg/ml	1 MHz, 0.5 W/cm², 70% cycle, 10 min	*In vitro*: 99.9% *In vivo*: 50 times lower than the control group	Triple therapy OAC	Equality	[32]
PPAF	*In vitro*: 200 μg/mL *In vivo*: 30 mg/kg	1 MHz, 1 W/cm², 50% cycle, 2 min	*In vitro*: 99% *In vivo*: 10 times lower than the US group	Triple therapy OAC	Antibiotics better	[77]
ICG@FCS	*In vitro*: 200 μg/mL *In vivo*: 30 mg/kg	1 MHz, 1.5 W/cm², 10 min	*In vitro*: 99% *In vivo*: 10 times lower than the US group	Triple therapy OAC	SDT better	[83]

* Triple therapy dose: omeprazole: 400 μmol/kg, amoxicillin: 28.5 mg/kg, clarithromycin:14.3 mg/kg.

the human stomach and is associated with various gastric diseases, including gastritis, peptic ulcers, and gastric cancer; its increasing resistance to traditional antibiotics and the disruption of the intestinal microbiota have heightened the urgency for novel therapeutic strategies, as HP infection is a predominant contributor to the development of gastric cancer. Extensive research has demonstrated that HP infection is the most significant and modifiable risk factor for gastric cancer prevention. Consequently, eradication of HP infection is recommended as the primary preventive intervention for gastric cancer. SDT, a nonantibiotic treatment modality, has the potential to manage HP infections by leveraging acoustic sensitizers to generate ROS under US stimulation and thereby eliminate pathogenic microorganisms [104]. This review delves into the application of SDT in treating HP infections and scrutinizes its potential for preserving the intestinal microbiota. Evidence suggests that SDT not only mitigates HP infections but also minimizes perturbations to the intestinal microbiota [32], potentially due to the localized and transient nature of ROS production. This attribute is vital for preserving gut health and averting antibiotic-related diseases.

While SDT has demonstrated substantial potential in experimental studies, several challenges must be addressed to enable its transition into the clinic. Future research should concentrate on the following pivotal areas: (1) Optimization of acoustic sensitizers to enhance their stability, targeting capabilities, and ROS production efficiency *in vivo*. This may involve chemical modifications to existing sensitizers and the creation of new nanomaterials to serve as carriers for acoustic sensitizers; (2) Preclinical studies: Additional *in vitro* and *in vivo* experiments are necessary to confirm the therapeutic efficacy of SDT across diverse HP infection models. This includes evaluating the safety, efficacy, and treatment windows for SDT. (3) Integration of treatment strategies: Investigating the potential for combining SDT with other therapeutic approaches, such as immunotherapy, could enhance treatment outcomes and prevent the development of drug resistance.

In conclusion, SDT-assisted biomaterials are a groundbreaking nonantibiotic therapy with promise to treat HP infections. By further studying its antimicrobial mechanisms and impacts on the gut microbiota, SDT is poised to become a treatment that is safe, effective, and compatible with the gut microbiome. After continued research and clinical trials, SDT can revolutionize the treatment landscape of HP infections, offering patients superior therapeutic options.

CRediT Author Statement

Mengfan Li: writing original draft, writing–review, editing, and investigation. **Yingli Gong and Tielou Chen**: writing original draft, writing–review, editing, and investigation. **Lei Lu, Xiuwen Ding, and Cuimin Chen**: making tables and figure. **Yan Wu, Tinglin Zhang, and Jie Gao**: supervision, resources, funding acquisition, writing–review, and editing.

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Conflict of Interests

The authors declare that no competing interest exists.

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