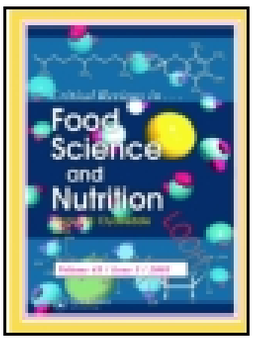

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REVIEW

Sialic acid-based strategies for the prevention and treatment of *Helicobacter pylori* infection: Emerging trends in food industry

Xiaohong Sun[a,b], Songyuan Zhang[a], Jian Ren[a], and Chibuike C. Udenigwe[b,c]

[a]College of Food and Biological Engineering, Qiqihar University, Qiqihar, Heilongjiang, China; [b]School of Nutrition Sciences, Faculty of Health Sciences, University of Ottawa, Ottawa, Ontario, Canada; [c]Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa, Ontario, Canada

ABSTRACT

Approximately 50% of the world population is infected with *Helicobacter pylori*. Antibiotics are widely used for *H. pylori* infection treatment but there are drawbacks, e.g., the emergence of antibiotic-resistant bacteria. Sialic acids are a family of acylated derivatives of a nine-carbon carboxylated monosaccharide. Because sialic acid of the host cells is vital to *H. pylori* pathogenesis, sialic acid-guided therapies have been proposed for the prevention and treatment of *H. pylori* infection, including anti-adhesive therapy and site-specific delivery. This review aims to shed light on the prospects of sialic acid-based strategies in the food industry for developing functional foods with potent anti-*H. pylori* activity. In this work, progress on the identification of sialic acid-containing components as anti-adhesive agents against *H. pylori* is reviewed. The current applications of sialic acid-based delivery systems in eradicating *H. pylori* are discussed, including microspheres, beads, hydrogels, and nanoparticles. The challenges and future perspectives of sialic acid-guided strategies and the possibility of their applications in food industry are highlighted. Antibiotic resistance is still a major challenge and the sialic acid-based technologies have tremendous potential to be utilized to develop functional foods that hold promise to be a future trend for preventing or treating *H. pylori* infection.

KEYWORDS

Sialic acid; *Helicobacter pylori*; anti-adhesive therapy; chitosan; mucoadhesive delivery system; functional food

Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative, spiral, microaerophilic, and highly motile bacterium that is generally identified in the antrum and cardia of the human stomach (Marshall and Warren 1984). It is estimated that approximately 50% of the world population is infected with *H. pylori*, and the prevalence could be as high as 80% in the developing countries (Smith, Fowora, and Pellicano 2019). *H. pylori* infection is closely associated with various gastric diseases, such as inflammation, chronic gastritis, peptic ulcers, mucosa-associated lymphoid tissue lymphoma, and gastric cancer, which give rise to high morbidity and mortality every year (Neshani et al. 2019; Walker and Crabtree 1998). It was reported that gastric cancer and peptic ulcers cause more than one million deaths per year (Axon 2014). *H. pylori* was ranked as a class I carcinogen by the World Health Organization (WHO) in 1994, and *H. pylori* infection is reported to be responsible for 75% of all gastric adenocarcinoma cases (De Martel et al. 2012).

The conventional approach to treat *H. pylori* infection is by using antibiotic regimens, which commonly include proton pump inhibitors, two antibiotics, and bismuth salt (Klesiewicz et al. 2018). The eradication rates of *H. pylori* by antibiotic therapy vary from 60% to 90%, but this is markedly compromised by the emergence of antibiotic-resistant strains (Goderska, Pena, and Alarcon 2018; Hu, Zhu, and Lu 2017). Besides, additional drawbacks of antibiotics include their degradation in the gastric environment, gastrointestinal side effects, poor water solubility, and low bioavailability (Cong et al. 2019). Based on a recent WHO report, the post-antibiotic era is approaching, in which antibiotic treatment would be ineffective and common bacterial infections can cause mortality (WHO 2014).

Antibiotic therapy is the most widely used treatment for *H. pylori* infection. However, its drawbacks have motivated researchers to develop alternative or improved approaches, such as the use of probiotics, vaccine, non-pharmaceutical anti-microbial therapy, stomach-specific drug delivery system, and anti-adhesive therapy (Fahey, Stephenson, and Wallace 2015; Malekshahi et al. 2011; Messing et al. 2014; O'Morain et al. 2018; Pan-In et al. 2014). Many of these prophylactic and therapeutic strategies have been developed based on *H. pylori* pathogenesis. As shown in Figure 1, when entering the stomach lumen, *H. pylori* first adheres to the mucus layer lining the gastric epithelium; this is an indispensable step in the establishment of an infection

CONTACT Xiaohong Sun x.sun xs7@ualberta.ca, sunxiaohong55@hotmail.com College of Food and Biological Engineering, Qiqihar University, Qiqihar, Heilongjiang, 161006, China; Chibuike C. Udenigwe cudenigw@uottawa.ca School of Nutrition Sciences, Faculty of Health Sciences, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada

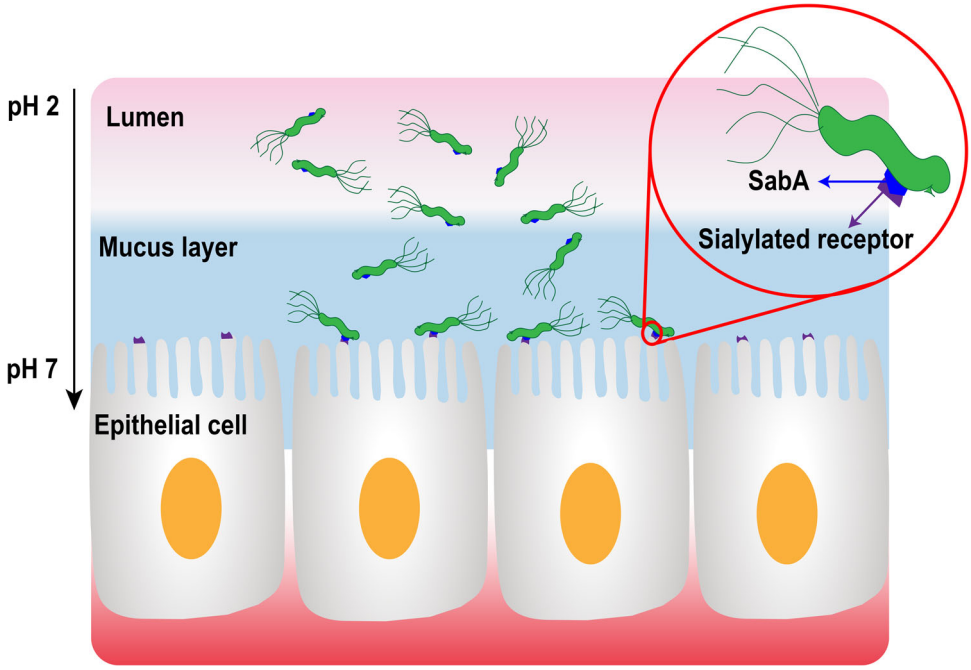

Figure 1. An overview of the pathogenesis of *H. pylori* infection.

(Magalhães and Reis 2010). Moreover, *H. pylori* utilizes urease to neutralize the acidic condition of the stomach and thus can survive in the harsh environment. Thereafter, *H. pylori* penetrates the mucus layers and moves toward the epithelial cells using their flagella. Once adhered to the epithelial surface, *H. pylori* can resist the forces exerted by gastric peristalsis and emptying, acquire nutrients for growth, deliver toxins to damage the host cells, and subsequently cause infection in the host (Isaeva and Fagoonee 2018). Specifically, the outer membrane proteins functioning as bacterial adhesins specifically recognize the glycosylated receptors on the host cell surface, achieving the *H. pylori* adhesion to gastric mucosa and epithelium (Amieva and El-Omar 2008).

Sialic acid on the surfaces of the host cells acts as a crucial binding site for *H. pylori*, and sialic acid-binding adhesin (SabA) of *H. pylori* is one of the most studied adhesins. Hence, various attempts have been made to develop sialic acid-based strategies for the prevention and treatment of *H. pylori* infection, mainly including anti-adhesive therapy and site-specific drug delivery system (Kao, Sheu, and Wu 2016; Varki 2008). The rapid research progresses have provided tremendous opportunities for the food industry to design and identify effective functional foods and nutraceuticals against *H. pylori* infection. To date, some food components have showed anti-*H. pylori* activity *in vitro*, such as polyphenolic compounds (e.g., curcumin) and essential oils, and few of them demonstrated strong anti-*H. pylori* activity in human clinical trials (Takeuchi et al. 2014). This article provides an overview of sialic acid-guided therapies and discusses the challenges and future perspectives, including potential use of the technology in the food industry for developing commercial functional foods and nutraceutical products with potent anti-*H. pylori* activity.

Roles and compositions of the gastric mucus

The gastric mucus, a viscous gel covering the entire gastric mucosa, is synthesized and released from the surface epithelial cells (Kauffman 1981). The continuous mucus layer acts as a physical barrier that protects the underlying gastric epithelium from damage by detrimental compounds in the lumen, such as corrosive acid, destructive hydrolases, and pathogens. At the same time, the mucus provides specific binding sites for bacterial adhesins, which enable bacteria to reside within the mucus (Atuma et al. 2001). For example, *H. pylori* particularly resides in the gastric mucus layer where they cause infection and gastric diseases.

The mucus is composed of approximately 95% water, 3% mucin, and 2% other compounds including salts, lipids, and proteins. Mucin is believed to be responsible for the protective function and viscoelastic property of the gastric mucus (Bansil and Turner 2006; Celli et al. 2005). Mucin is a large glycoprotein with molecular weight ranging from 0.5 to 20 MDa. Mucin adopts a complex bottle-brush configuration with glycans as the side chains attached to the protein core. Human mucin is highly glycosylated and consists of ~80% carbohydrates, including *N*-acetylglucosamine, *N*-acetylgalactosamine, fucose, galactose, sialic acid (*N*-acetylneuraminic acid, Neu5Ac), and traces of mannose and sulfate (Bansil and Turner 2006). Due to the presence of terminal sialic acid and sulfate, most mucins carry a net negative charge at the acidic pH of the stomach (Khutoryanskiy 2011).

Sialic acid (Neu5Ac): an important monosaccharide at the terminal position of the glycan chains of mucin

Sialic acids are a family of acylated derivatives of a nine-carbon carboxylated monosaccharide (Yu et al. 2004). With

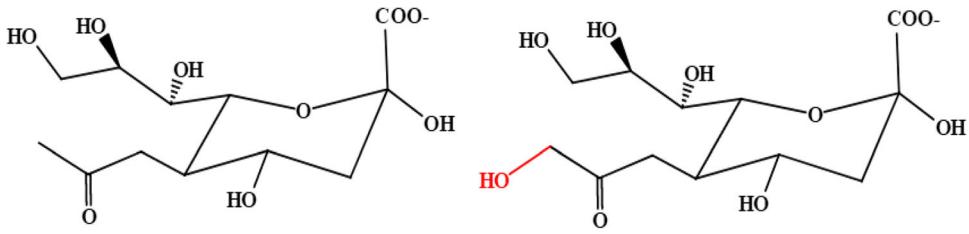

Figure 2. Structures of two most abundant forms of sialic acids, *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc).

more than 50 derivatives identified to date, the most abundant forms of sialic acids are Neu5Ac and *N*-glycolylneuraminic acid (Neu5Gc), as shown in Figure 2. Neu5Ac is the only form of sialic acid found in healthy humans, and Neu5Gc is present in many animal species and human cancer cells and considered a non-human sialic acid (Wang and Brand-Miller 2003).

Neu5Ac is rarely present in free form in nature. It commonly acts as a vital component of oligosaccharide chains of mucins, glycoproteins, and glycolipids, and usually occupies the terminal, non-reducing positions of glycan chains (Wang and Brand-Miller 2003). Sialic acid plays important roles in many biological processes, such as acting as recognition sites for microorganisms, toxins and hormones, protecting cells from enzymatic hydrolysis and immunological attacks, preserving the integrity of the mucin structures, and involving in intermolecular and intercellular interactions (Spichtig, Michaud, and Austin 2010). This review focused mainly on the function of sialic acid at the terminal position of glycan chains of the gastric mucin as the recognition site for *H. pylori*.

Sialic acid binding property of *H. pylori* mediated by SabA

H. pylori recognizes the sialylated receptors present in gastric mucin and epithelial cells via SabA (Dunne, Dolan, and Clyne 2014). SabA is one of the most well-characterized and dominant *H. pylori* adhesins and was isolated by the retagging method based on its affinity for sialyl-Lewis^x (Mahdavi et al. 2002). SabA is an outer membrane protein with molecular weight of 66 kDa (Dunne, Dolan, and Clyne 2014). The three-dimensional structure of the soluble extracellular adhesion domain of SabA was determined by X-ray crystallography and shown to exhibit distant similarity to the tetratricopeptide repeat fold family (Pang et al. 2014).

SabA preferentially binds to the sialyl-Lewis^x antigen and the required minimal binding structure is the Neu5Acα2-3Gal-disaccharide. SabA binding to the sialyl- Lewis^x epitope expressed in the gastric mucosa could enhance *H. pylori* colonization, and promote chronic infection and inflammatory processes in the gastroduodenal tract (Fagoonee and Pellicano 2019). Therefore, SabA is an important virulence factor for the pathogenesis of *H. pylori* infection.

Prevention of *H. pylori* infection by anti-adhesive therapy based on sialic acid-containing compounds

H. pylori adhesion to the specific receptors on the gastric mucins and epithelium is considered a prerequisite initial step in the infection process (Magalhães and Reis 2010). As discussed earlier, *H. pylori* adhesion to gastric mucins and epithelium is mediated by adhesins, and SabA is one of the predominant adhesins that is responsible for recognizing and binding to the sialylated receptors on the host cell surfaces. Thus, anti-adhesive therapy has been designed to interfere with or inhibit *H. pylori* adherence to the gastric mucosa and epithelial cells via SabA and subsequently prevent *H. pylori* infection.

Anti-adhesive therapy is regarded as an alternative approach to antibiotic treatments. The proposed mechanism of anti-adhesive therapy against *H. pylori* based on sialic acid-containing compounds involves blocking the interactions between SabA and sialylated receptors using sialic acid-containing components as receptor analogs (Figure 3). Compared to antibiotic regimens, anti-adhesive therapy has many advantages, such as its mildness, safeness, and low rate of producing drug-resistant strains (Karlsson 1998; Ofek, Hasty, and Sharon 2003).

In this context, much research effort has been devoted to identifying and developing sialic acid-containing components as anti-adhesive agents to interfere with *H. pylori* adhesion via SabA. Most of the identified sialic acid-containing components with anti-adhesive activity against *H. pylori* are naturally present in human and bovine milk, which has been summarized in Table 1. In the late 1990s, researchers started to determine the anti-adhesive activity of sialic acid-containing oligosaccharides and glycoconjugates against *H. pylori* binding to gastrointestinal epithelial cells, such as α_1-acid glycoprotein, fetuin, porcine gastric and bovine submaxillary mucins, glycolipid sulfatide, and 3′-sialyllactose sodium salt (3′-SL) (Simon et al. 1997). 3′-SL, an oligosaccharide naturally occurring in human and bovine milk, was demonstrated to be the most-active anti-adhesive oligosaccharide (Simon et al. 1997). Based on the *in vitro* results, the safety and efficacy of 3′-SL for eradication of *H. pylori* were evaluated in rhesus monkeys (Mysore et al. 1999). This work proved the safety of anti-adhesive therapy based on 3′-SL although its efficacy was inconclusive because 2 of 6 monkeys were cured permanently and one was transiently cleared after treatment with 3′-SL. However, this therapy was not effective in the other animals, which remained

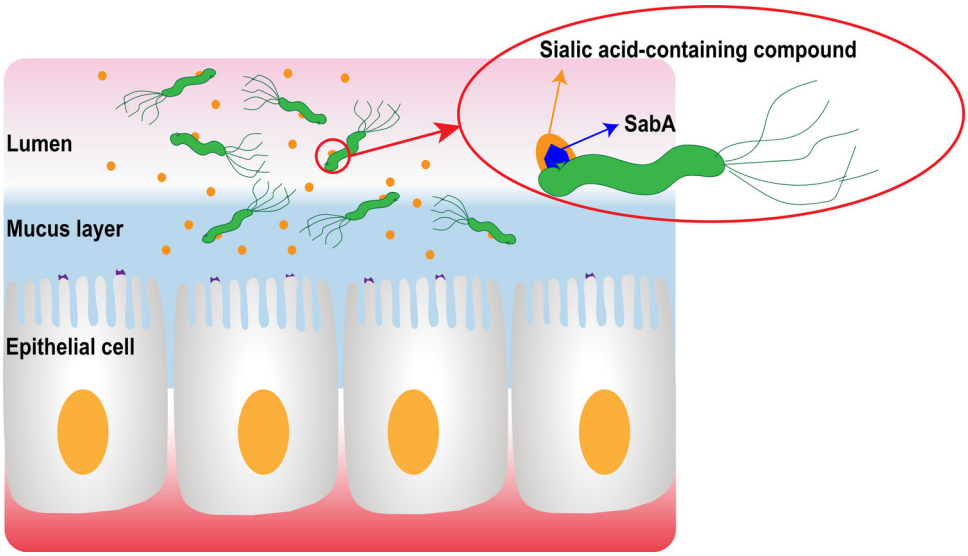

Figure 3. Mechanism of anti-adhesive therapy in the prevention of *H. pylori* infection. Sialic acid-containing compounds act as host receptor analogs to competitively bind *H. pylori* via SabA and subsequently prevent *H. pylori* adhesion to epithelial cells.

Table 1. Identification of sialic acid-containing compounds as anti-adhesive agents against *H. pylori*.

Sialic acid-containing compound	Dietary constituent	Phases of clinical research	Possibility to be used in food industry[a]	Reference (year)
α1-Acid glycoprotein	No	*In vitro*	Low	Simon et al. (1997)
Fetuin	No	*In vitro*	Low	
Porcine gastric mucin	No	*In vitro*	Low	
Bovine submaxillary mucin	No	*In vitro*	Low	
Glycolipid sulfatide	No	*In vitro*	Low	
3′-Sialyllactose sodium salt	Yes	*In vitro*, animal study, clinical trail	High	Simon et al. (1997); Mysore et al. (1999); Parente et al. (2003)
Lactoferrin	Yes	Animal study	High	Wang et al. (2001)
Fat globule membrane fractions	Yes	Animal study	High	Wang et al. (2001)

aEvaluated by the source of anti-adhesive compounds.

persistently colonized by *H. pylori* after the treatment. In a double-blind, placebo-controlled clinical study, the safety of the 3′-SL-mediated anti-adhesive therapy was validated in humans but 3′-SL did not reduce or eradicate *H. pylori* colonization (Parente et al. 2003). In addition, sialylated glycoconjugates from bovine milk, lactoferrin, and fat globule membrane fractions showed tremendous potential in inhibiting *H. pylori* infection in a BALB/cA mouse model. Compared to the *H. pylori*-infected control group, both glycoconjugates significantly reduced *H. pylori* colonization and decreased inflammation scores of the treated mice (Wang et al. 2001).

Although several sialic acid-containing compounds showed potent anti-adhesive activity against *H. pylori*, identification of more effective, low-cost, readily available anti-adhesive agents is still indispensable for the development of functional foods and nutraceuticals. Bovine milk and chicken egg white are promising dietary sources of anti-adhesive agents against *H. pylori*. Despite the prospects, the molecular mechanisms of the anti-adhesive agents need to be elucidated, including validation of the interaction between sialic acid-containing compounds and *H. pylori* via SabA, evaluating the possible influencing factors, understanding the underlying driving forces, and analysis of the kinetics of such molecular interaction. Sialic acid is indispensable for the anti-adhesive activity against *H. pylori*; the anti-adhesive activity significantly decreased after treatment of the glycoconjugates with sialidase (Hirmo et al. 1998; Parker et al. 2010). However, the relationship between anti-adhesive activity and glycan structures is still unclear. To our knowledge, only one study reported that the anti-adhesive activity of sialic acid-containing glycoconjugates against *H. pylori* did not correlate with the sialic acid content. The authors speculated that the glycan structures, number of sugar chains, and the position of sialic acid may have played the major roles instead (Matsumoto et al. 2005). Thus, the structure–activity relationship of the anti-adhesive compounds is another future research direction in this field.

Treatment of *H. pylori* infection using sialic acid-based delivery systems: chitosan or its derivatives-based mucoadhesive delivery systems

Drug or bioactive compound delivery systems have been extensively applied in the pharmaceutical and food industries to achieve controlled, stable, and site-specific delivery (Mohan et al. 2015). Different types of drug delivery systems have been investigated to overcome the challenges of utilizing antibiotics in treating *H. pylori* infection, including low stability and bioavailability, short residence time, and poor

water solubility (Lopes et al. 2014). Non-mucoadhesive drug delivery systems are incapable of increasing the contact time and enhancing the effective drug concentration at the *H. pylori* infection site due to their inability to withstand peristalsis and the washing effect of the stomach (Ways et al. 2018). In contrast, mucoadhesive drug-delivery systems can adhere to the gastric mucosa at sites of *H. pylori* colonization, increase the residence time of drugs, achieve sustained drug release, and enhance bioaccessibility (Ways et al. 2018). Various mucoadhesive polymers have been fabricated to display distinct features, such as strong hydrogen bonding, negatively or positively charged groups, high-molecular weight, and chain flexibility. Many theories have been proposed to explain the mucoadhesion between the developed polymers and mucins, such as the electronic, adsorption, wetting, and diffusion theories; however, the adhesion mechanism is still not fully understood (Khutoryanskiy 2011).

Chitosan is one of the most extensively used polymers for producing mucoadhesive drug-delivery systems due to its non-toxicity, non-immunogenicity, antimicrobial activity, availability, and mucoadhesive properties (Ways et al. 2018). Chitosan is a linear and cationic polysaccharide composed of D-glucosamine and *N*-acetyl-D-glucosamine residues, and is prepared by alkaline deacetylation of chitin (Ways et al. 2018). Nonetheless, chitosan is highly soluble in acidic conditions where it loses its three-dimensional structure. This property will result in a fast drug release in the stomach, which limits the application of chitosan as a gastro-mucoadhesive delivery system (Gonçalves et al. 2014). To overcome the problem, the use of crosslinking agents and generation of chemically modified chitosan derivatives have been widely explored. Therefore, mucoadhesive antibiotic delivery systems based on chitosan and its derivatives are the most commonly adopted sialic acid-based strategies for treating *H. pylori* infection. This approach has a tremendous potential to be applied in the food industry for developing functional foods with anti-*H. pylori* activity. In addition, the electrostatic interaction between the cationic free amines of chitosan and anionic sialic acid of mucin at acidic pH in stomach is believed to play a crucial role in the mucoadhesive properties of chitosan (Gonçalves et al. 2014; Qaqish and Amiji 1999; Ways et al. 2018). The representative sialic acid-based delivery systems for *H. pylori* eradication were discussed in section "Types of Mucoadhesive Delivery Systems Based on Chitosan or its Derivatives" and listed in Table 2.

Interactions between chitosan and mucin

In order to optimize mucoadhesion, increase drug bioavailability, improve therapeutic efficacy, and develop potent chitosan-based mucoadhesive drug or nutraceutical delivery systems against *H. pylori* infection, many research efforts have been made to understand the mucoadhesive mechanisms and molecular basis of the chitosan–mucin interaction. Based on turbidimetric and mucin adsorption assays, the interactions between chitosan and mucin in water were found to be dominated by electrostatic interaction because the surface charges of chitosan and mucin showed a remarkable influence on mucoadhesion (He, Davis, and Illum 1998). Moreover, sialic acid was reported to play an essential role in the electrostatic interaction, which was positively correlated with the strength of electrostatic interaction (He, Davis, and Illum 1998). In addition, based on fluorescence polarization assay at different pH and ionic strengths, hydrogen bonding and hydrophobic interaction were found to also contribute significantly to the mucin–chitosan interaction (Qaqish and Amiji 1999).

The interaction of chitosan and mucin was profoundly affected by their structural features, such as the sialic acid content of mucin and degree of acetylation, molecular weight, and acetylation pattern of chitosan (Collado-González, González Espinosa, and Goycoolea 2019; He, Davis, and Illum 1998). For example, chitosan with high-molecular weight was reported to interact with more mucins and possess a higher magnitude of interaction possibly because the chitosan molecule can bind to multiple mucin molecules (Menchicchi et al. 2014; Qaqish and Amiji 1999). Furthermore, the chitosan–mucin interactions are affected by external parameters, such as the pH and ionic strength of the system where the interaction occurs. For instance, a pH between 2.4 and 6.3 facilitates the chitosan–mucin interactions whereas high ionic strength of the solution reduces the interactions (Collado-González, González Espinosa, and Goycoolea 2019). It is well accepted that polymers with strong mucoadhesion are readily to be washed out upon mucus turnover, which may negatively impact their mucopenetrating properties (Gonçalves et al. 2014). The molecular nature of interactions between mucin and chitosan is complex and yet to be completely elucidated. To date, most of the published data were obtained from *in vitro* studies using commercially available mucins, such as porcine gastric mucin and bovine submaxillary mucin, which were different from the mucus layers found *in vivo*. Thus, future studies should use biologically relevant models to obtain more realistic evidence and mechanism of the chitosan–mucin interactions.

Types of mucoadhesive delivery systems based on chitosan or its derivatives

Microspheres

Microspheres are spherical particles with diameters in the range of 1–1000 µm. They are characterized as matrix systems where the encapsulated compound is homogeneously dispersed in either a solution or a suspension (Lengyel et al. 2019). The development of a stomach-specific mucoadhesive microsphere delivery system was first reported in the early 2000s. First, chitosan microspheres with an average diameter of 2–3 µm were manufactured by ionic crosslinking with sodium sulfate. Tetracycline was successfully loaded into the pre-formed chitosan microspheres, and the incorporated tetracycline was found to be stable for up to 12 h even at acidic pH (Hejazi and Amiji 2002, 2003). However, the chitosan microspheres did not reduce the drug release rate, prolong the gastric residence, or increase the tetracycline

Table 2. The representative sialic acid-based delivery systems for *H. pylori* eradication.

Formulation	Property	Phases of research	Anti-*H. pylori* effect[a]	Encapsulated compound	Possibility to be used in food industry[b]	Reference (year)
Microspheres						
Chitosan, sodium sulfate, Tween 80	Increased acidic stability	*In vitro, in vivo*	N.D.[a]	Tetracycline	High	Hejazi and Amiji (2002, 2003)
Chitosan, sodium sulfate, Tween 80, glyoxal	Increased drug concentration and retention in stomach	*In vivo*	N.D.[a]	Tetracycline	Low	Hejazi and Amiji (2004)
Chitosan, paraffin, dioctyl sodium sulfosuccinate, ethyl cellulose glutaraldehyde	Increased drug concentration and retention in stomach, sustained drug release, increased bioavailability	*In vitro, in vivo*	N.D.[a]	Clarithromycin	Low	Majithiya and Murthy (2005)
Chitosan, paraffin, dioctyl sodium sulfosuccinate, glutaraldehyde,	Increased gastric retention, sustained drug release	*In vivo*	Improve Anti-*H. pylori* effect	Amoxicillin	Low	Patel and Patel (2007)
Chitosan, pentasodium tripolyphosphate	Increased acidic stability, sustained drug release	*In vitro*	N.D.[a]	Ampicillin	High	Anal et al. (2006)
Chitosan, polymers (EC-10 or EC-46), polyvinyl alcohol, concanavalin A	Buoyancy, improved mucoadhesion	*In vitro*	N.D.[a]	Clarithromycin	Low	Adebisi and Conway (2014b)
Beads						
Chitosan, magnesium stearate, calcium alginate, polymer (methylcellulose, carbopol 934 P or κ-carrageenan)	Buoyancy, sustained drug release	*In vitro, in vivo*	Improve Anti-*H. pylori* effect	Metronidazole	High	Ishak et al. (2007)
Chitosan, gellan,	sustained drug release, strong mucoadhesion	*In vitro*	N.D.[a]	Amoxicillin	High	Narkar et al. (2010)
Chitosan, *Caesalpinia pulcherrima* galactomannan, sodium alginate	Buoyancy, increased gastric retention, sustained drug release, strong mucoadhesion	*In vitro, in vivo*	Potent anti-*H. pylori* effect	Amoxicillin	High	Thombre and Gide (2016)
Chitosan, sodium alginate, sunflower oil, hydroxypropyl methylcellulose	Buoyancy, increased gastric retention, sustained drug release, strong mucoadhesion	*In vitro*	Potent anti-*H. pylori* effect	Amoxicillin	High	Dey et al. (2016)
Hydrogels						
Chitosan, polyvinyl pyrrolidone, glutaraldehyde	Increased swelling property	*In vitro*	N.D.[a]	Amoxicillin	Low	Risbud et al. (2000)
Chitosan, poly (acrylic acid)	Increased gastric retention	Clinical trail	N.D.[a]	Amoxicillin	High	De la Torre et al. (2005)
Chitosan, poly (acrylic acid)	N.D.[a]	Clinical trail	No anti-*H. pylori*	Amoxicillin and Clarithromycin	High	Gisbert et al. (2006)
Nanoparticles						
Chitosan, heparin	stomach-specific and pH-responsive properties	*In vitro, in vivo*	N.D.[a]	None	High	Lin et al. (2009)
Chitosan, fucose, heparin, genipin	Sustained drug release, stomach-specific and pH-responsive properties	*In vitro, in vivo*	Improved anti-*H. pylori* effect	Amoxicillin	High	Lin et al. (2013)
Chitosan, 12-aminododecanoic acid, sodium tripolyphosphate	Sustained drug release, stomach-specific and pH-responsive properties	*In vitro*	Potent anti-*H. pylori* effect	Amoxicillin	Low	Jing et al. (2016)
Chitosan, sodium alginate,	Increased gastric retention	*In vitro, in vivo*	Improved anti-*H. pylori* effect	Antimicrobial Peptide Pexiganan	High	Zhang et al. (2015)
Chitosan, fucose, gelatin	Sustained release, stomach-specific property	*In vitro, in vivo*	Improved anti-*H. pylori* effect	Epigallocatechin-3-gallate (ECGG)	High	Lin et al (2014)

aNot determined.
bEvaluated by the formulation of delivery system.

concentration in gerbil stomach (Hejazi and Amiji 2002, 2003). To overcome these challenges, glyoxal-crosslinked chitosan microspheres were fabricated by chemical crosslinking that were resistant to acid dissolution (Hejazi and Amiji 2004). *In vivo* studies showed that, compared to tetracycline solution, the glyoxal-crosslinked chitosan microspheres remarkably enhanced the local tetracycline concentration in the gerbil stomach from 447.3 to 868.9 μg h/g tissue and increased the gastric residence time due to the electrostatic interactions between the chitosan microsphere and the sialic acid of mucin (Hejazi and Amiji 2004). Likewise, since glutaraldehyde is the most commonly used chemical

crosslinking agent, chitosan mucoadhesive microspheres have been fabricated by the emulsification technique using glutaraldehyde to encapsulate clarithromycin (Majithiya and Murthy 2005). However, these studies did not validate the efficacy of the chitosan microspheres in eradicating *H. pylori* infection *in vivo*. In a later study with *H. pylori* infected Wistar rats, glutaraldehyde crosslinked amoxicillin-loaded chitosan microspheres was found to have a better *H. pylori* clearance effect than the free drug (Patel and Patel 2007). Since glutaraldehyde can cause adverse health effects such as mucosal irritation, ionotropic crosslinked technique using pentasodium tripolyphosphate has been explored as an alternative. The pentasodium tripolyphosphate crosslinked chitosan microspheres showed a better stability in simulated gastric fluid and a controlled release of ampicillin compared to uncrosslinked microspheres (Anal, Stevens, and Remunan-Lopez 2006).

The nonspecific electrostatic interactions between mucin and chitosan are likely to be interfered by other competitive binding matrix such as food and shed mucus. Hence, lectin-conjugated mucoadhesive delivery systems, such as microspheres, have been explored because lectins can specifically bind to the carbohydrate moieties of gastric mucin and glycoconjugates on the surface of the epithelium (Adebisi and Conway 2011). For instance, concanavalin-A (Con A) was successfully conjugated to the surfaces of ethylcellulose/chitosan microspheres by two-stage carbodiimide activation (Adebisi and Conway 2014b; Jain and Jangdey 2009) and the amount of lectin conjugation ranged from 11 to 15 µg Con A/mg microspheres (Adebisi and Conway 2014b). The conjugation significantly improved mucoadhesion from 12% to 85% compared to the non-conjugated microspheres (Jain and Jangdey 2009). Taken together, mucoadhesive chitosan microspheres have been extensively investigated for increasing the gastric residence time and achieving a controlled release of therapeutic agents. However, these studies can only provide indirect evidence of the efficacy of the microspheres for *H. pylori* eradication. Future research should be shifted toward determining the amounts of the active compounds interacting locally at the region of *H. pylori* infection on the gastric epithelium, and evaluating the therapeutic effects of the chitosan microspheres against *H. pylori* infection in preclinical and clinical studies.

Beads

In some literatures, microspheres and beads are interchangeable, but in this review, beads refer to solid, spherical, and millimeter-sized carriers of drug or bioactive compounds. Beads with floating and mucoadhesive characteristics are prone to getting widely distributed along the gastrointestinal tract (Thombre and Gide 2016). To improve floating and drug entrapment efficiency and achieve a sustained release, chitosan-treated alginate beads were prepared by ionotropic gelation using methylcellulose, carbopol 934P, and κ-carrageenan as viscosity-enhancing polymers, chitosan as encapsulating polymer, and magnesium stearate as a floating aid. The bead formulation with the best performance, such as immediate buoyancy (100% of floating beads), high drug entrapment efficiency (92%), and controlled drug release ($T_{80\%}$ 215 min), was that derived from 0.5% κ-carrageenan, 0.4% chitosan, and 5% magnesium stearate as determined by a ($3 \times 2 \times 2$) factorial design experiment. The *in vivo H. pylori* clearance tests revealed that the prepared chitosan-treated beads provided 100% clearance rate at a once daily dose of 15 mg/kg for three consecutive days while the free drug showed only 33% clearance at a dose of 20 mg/kg (Ishak et al. 2007). Despite the strong prospects, long-term treatment studies are needed to confirm the complete *H. pylori* eradication in longer durations.

Similarly, beads have been fabricated using gellan gum and chitosan by ionotropic gelation method for loading acid-soluble drug. Besides the high drug entrapment efficiency (60%–90%) and controlled drug release (up to 7 h), the beads showed strong mucoadhesion where over 85% of beads maintained their interactions with the stomach mucosa of albino rats after 7-h post-administration (Narkar, Sher, and Pawar 2010). When using *Caesalpinia pulcherrima* galactomannan as viscosity-imparting polymer, the chitosan-based beads showed 79%–92% drug release, 65–89 entrapment efficiency, and 61%–89% mucoadhesion (Thombre and Gide 2016). Further, the efficacy of the prepared beads on the *H. pylori* eradication was investigated *in vivo* using *H. pylori* infected male Wistar rats at the end of 4th, 7th, and 10th week. The clearance rates of developed beads containing amoxicillin at 4th, 7th, and 10th week were 67%, 83%, and 100%, respectively. The authors speculated the potent anti-*H. pylori* effects of prepared beads were attributed to the prolonged gastric residence time owing to their floating and mucoadhesive characteristics (Thombre and Gide 2016). Moreover, the bead formula was modified by the addition of oil to improve buoyancy (Adebisi and Conway 2014a; Adebisi, Laity, and Conway 2015). The sunflower oil entrapped inside the floating and mucoadhesive beads that were composed of sodium alginate and hydroxypropyl methylcellulose as matrix polymers and chitosan as coating polymer was confirmed by scanning electron microscopy. The beads containing amoxicillin trihydrate floated for more than 24 h and exhibited 100% *H. pylori* growth inhibition within 15 h *in vitro* (Dey et al. 2016). The anti-*H. pylori* efficacy of this novel bead need to be validated *in vivo*.

Hydrogels

Hydrogels are networks of polymer chains extensively swollen in aqueous medium that form a tangled mesh structure, functioning as a matrix for drug or nutraceutical entrapment and delivery (Ahmed 2015; Risbud et al. 2000). Risbud et al. (2000) synthesized a pH-sensitive hydrogel by crosslinking chitosan and polyvinyl pyrrolidone with glutaraldehyde for antibiotic delivery. Moreover, porous freeze-dried hydrogels and non-porous air-dried hydrogels were fabricated to compare their swelling and drug-release properties. Due to the porous nature, the freeze-dried hydrogels had a significantly higher swelling over the tested pH values (1.0, 2.0, 3.0, 4.0, 7.0, and 9.2) and faster amoxicillin release rates at pH 1.0–3.0 compared to the air-dried hydrogels. However, the authors concluded that the rapid release profile of the

freeze-dried hydrogels as drug-delivery systems was desirable to drug efficacy, which is conflicting with the current findings that the sustained release is preferable. The prolonged gastric retention (up to 3 h) of chitosan-based hydrogels have been demonstrated by *in vivo* studies in healthy humans (De la Torre, Torrado, and Torrado 2005; Torrado et al. 2004). Unfortunately, chitosan-based hydrogels incorporating 100 mg of amoxicillin or 50 mg of clarithromycin which were both administered twice daily for 7 days did not improve the anti-*H. pylori* efficacy in 40 patients with peptic ulcer or functional dyspepsia (Gisbert et al. 2006). This lack of effect may be because of the relatively rapid drug releasing property of hydrogels.

Nanoparticles

The microparticle delivery systems are able to prolong the gastric retention time of drugs by mucoadhesion, as discussed above. However, they may not provide sufficient drug concentrations at *H. pylori* infection sites because they usually have limited penetration in the gastric mucosa due to the micrometre-scale sizes, making them more likely to be shed off upon mucus turnover (Figure 4a) (Date, Hanes, and Ensign 2016). Mucoadhesive nanoparticle carrier systems can overcome these challenges. Nanoparticles have dimensions usually smaller than 100 nm in length and are more likely to penetrate through the mucus layers and reach the gastric epithelial surface where *H. pylori* colonizes (Madureira, Pereira, and Pintado 2015).

To date, substantial efforts have been made to develop mucoadhesive antibiotic-loaded nanoparticles, such as amoxicillin and clarithromycin-based chitosan–glutamate nanoparticles, to increase patient compliance, improve efficacy, and minimize dose-related adverse effects of antibiotics (Ramteke et al. 2009). Special attention has been devoted to the preparation of multifunctional antibiotic-loaded nanoparticles, which could protect antibiotics from destruction in the gastric fluid, adhere to the gastric mucosa, prolong the residence time, and penetrate into the mucus layer. Moreover, multifunctional nanoparticles can directly interact with or specifically target *H. pylori* on the gastric epithelium and subsequently release antibiotics at the site of *H. pylori* infection due to its pH sensitivity (Figure 4b) (Lin et al. 2013). The hypothesis of the multifunctional nanoparticle was first tested using a pH-responsive chitosan/heparin nanoparticle that was stable at pH 1.2–2.5 but became unstable at pH 7.0 where it disintegrated and released the loaded drug. Furthermore, these nanoparticles were found to interact locally at the *H. pylori* infection sites by nonspecific binding (Lin et al. 2009). Conjugated nanoparticles based on *H. pylori* receptors have also been designed to specifically recognize and target the region of *H. pylori* infection on the gastric epithelium. This system can prevent the disruption of the intestinal flora and reduce the emergence of antibiotic resistance. For instance, genipin-crosslinked fucose-chitosan/heparin nanoparticles were designed for amoxicillin encapsulation, with the fucose receptor serving as the recognition site for *H. pylori*. The components of this nanoparticle exerted distinct functions: chitosan and heparin were important for pH responsiveness; genipin was used as a natural crosslinker of the amine groups to improve the nanoparticle stability and control the drug release rate; and fucose was used to target *H. pylori* on the gastric epithelium (Lin et al. 2013). Using this system, the release of amoxicillin from the genipin-crosslinked nanoparticles decreased by ~61% and 67% within 120 min at pH 1.2 and 6.0, respectively, when compared with the non-genipin-crosslined nanoparticles. Furthermore, the genipin-crosslinked nanoparticles had a rapid drug release at pH 7.0 due to structure collapse. *In vivo*, the genipin-crosslinked fucose-chitosan/heparin nanoparticles significantly improved the anti-*H. pylori* effect (~73%) compared with the amoxicillin solution, possibly because of their specific targeting and active contact with *H. pylori* (Lin et al. 2013). Likewise, the ureido-conjugated chitosan/sodium tripolyphosphate (TPP) multifunctional nanoparticles were proposed for targeted amoxicillin delivery for *H. pylori* eradication. This targeting system works because of the presence of urea transport protein (Urel) on the membrane of *H. pylori* that specifically recognizes urea. Compared to unmodified amoxicillin–chitosan/TPP nanoparticles, the ureido-conjugated nanoparticles provided a more favorably sustained drug release, and more specific and enhanced anti-*H pylori* efficacy *in vitro* (Jing et al. 2016). Further study demonstrated that the uptake of the ureido-conjugated nanoparticles into *H. pylori in vitro* was dependent on the pH, temperature, and the addition of a competitive substrate, urea. Compared with unmodified nanoparticles, the ureido-conjugated nanoparticles showed a stronger *in vivo* anti-*H. pylori* efficacy using Balb/C male mice as the *H. pylori*-infected model (Jing et al. 2018). These studies indicated the multifunctional antibiotic-loaded nanoparticles hold strong promise for the effective treatment of *H. pylori* infection. Antimicrobial peptides and phenolic compounds, as antibiotic alternatives, have been successfully encapsulated within mucoadhesive nanoparticles to improve their anti-*H. pylori* activity. For example, chitosan–alginate nanoparticles loaded with antimicrobial peptide, pexiganan, were generated by the ionic gelation method and found to have a longer gastric residence time and more effective eradication of *H. pylori* from the stomach of *H. pylori*-infected rats and mice, compared wih free pexiganan (Zhang et al. 2015). In addition, fucose–chitosan/gelatin nanoparticles were developed as efficient nanocarriers for the delivery of epigallocatechin-3-gallate (EGCG), a dominant polyphenol in green tea. The nanoparticles effectively controlled the EGCG release in a simulated gastrointestinal fluid *in vitro* with cumulative EGCG release of ~26%, 40%, and 80% at pH 2.5, 6.0, and 7.4, respectively, within 120 min at 37 °C (Lin et al. 2014). *In vivo* study showed that the EGCG nanoparticles increased the *H. pylori* eradication rate by ~30% compared with EGCG solution in gastric-infected mice (Lin et al. 2014). However, the development of sialic acid-based nanoparticle delivery systems for the antibiotic alternatives is still in the early stage. Furthermore, future studies should place more emphasis on the application of food-derived compounds as antibiotic alternatives.

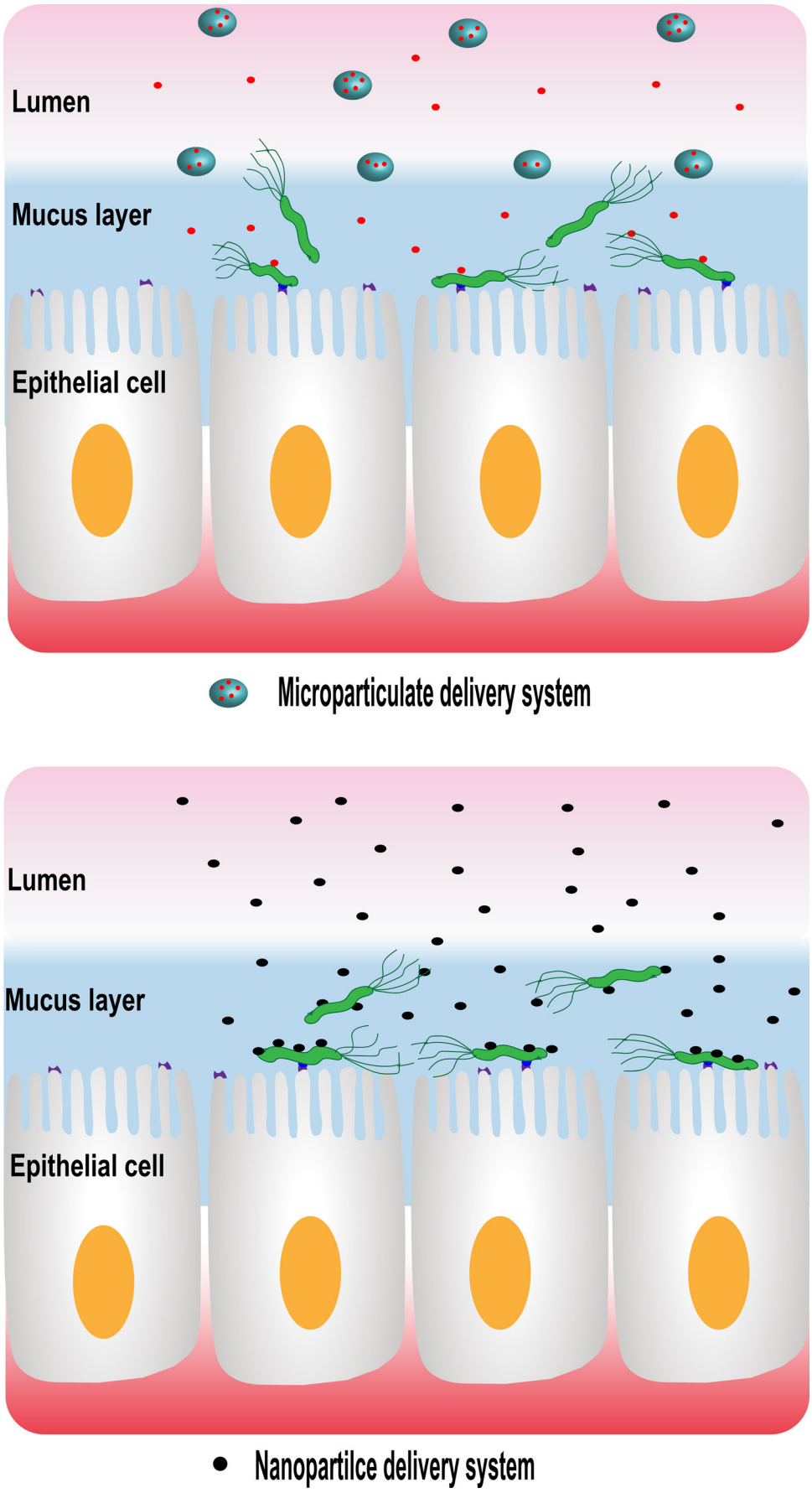

Figure 4. (a) Schematic diagram of treatment of *H. pylori* infection by mucoadhesive microparticulate delivery systems. (b) Schematic diagram of treatment of *H. pylori* infection by mucoadhesive nanoparticle delivery systems.

Conclusion and future perspectives

To overcome the drawbacks of antibiotics, anti-adhesive therapy has been designed to inhibit *H. pylori* adherence to host cells and subsequently prevent *H. pylori* infection. Notably, food-derived components containing sialic acid have demonstrated potent anti-adhesive activity against *H. pylori in vitro*, and can be further explored are promising ingredients for producing functional foods and nutraceuticals. Future research efforts should be concentrated on the identification of more effective, inexpensive, and readily available anti-adhesive agents especially from food sources such as bovine milk and chicken egg white. In addition, the molecular anti-adhesive mechanisms of sialic acid-containing compounds against *H. pylori* need to be unraveled.

Sialic acid-based delivery systems, especially mucoadhesive delivery systems based on chitosan or its derivatives, have been proposed for overcoming the limitations of antibiotics. The microparticle delivery systems are able to prolong gastric residence time by mucoadhesion, and microspheres were the most well-studied carriers for antibiotic delivery. Due to their limited mucosa penetrating property, the microparticle vehicles may not sufficiently deliver drug at sites of *H. pylori* infection. Nanoparticle formulations, particularly multifunctional nanoparticles, demonstrated better properties in preventing antibiotics degradation, adherence to gastric mucosa, prolonging the retention time, penetration into mucus layer, specific targeting of *H. pylori*, and releasing the loaded antibiotics at the region of *H. pylori* infection. Currently, site-specific nanoparticle delivery systems are developed largely at the preclinical stage. Therefore, the efficacy of the nanoparticles in eradicating *H. pylori* still needs to be validated in infected humans. Generation of sialic acid-conjugated nanoparticles that specifically recognize *H. pylori* may contribute in enhancing the anti-*H. pylori* activity.

Although sialic acid-based delivery systems could overcome some drawbacks of antibiotics and improve anti-*H. pylori* effects, antibiotic resistance is still a major challenge. This highlights the need for research in the development of antibiotic alternatives. Antimicrobial peptides and phenolic compounds have been successfully loaded into mucoadhesive nanoparticles to improve their anti-*H. pylori* activity, although this research field is still in its infancy. The sialic acid-based technology discussed in this review offers good promise in the food industry as functional foods and nutraceuticals have tremendous potential to represent the future trends in preventing or treating *H. pylori* infection.

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ORCID

Xiaohong Sun http://orcid.org/0000-0002-2346-0347
Chibuike C. Udenigwe http://orcid.org/0000-0001-8802-7707

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