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Preparation and evaluation of amoxicillin loaded dual molecularly imprinted nanoparticles for anti-*Helicobacter pylori* therapy

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ABSTRACT

This paper reports studies on preparation and evaluation of amoxicillin loaded dual molecularly imprinted nanoparticles (Amo/Dual-MIPs) designed for anti-*H. pylori* therapy. Both MNQA and AmoNa were chosen as templates to prepare Dual-MIPs using inverse microemulsion polymerization method. NQA was modified with myristic acid (MNQA) to become amphiphilic and assist in leaving NQA cavities on the surface of Dual-MIPs for *H. pylori* adhesion. AmoNa was applied to produce imprinting sites in Dual-MIPs for rebinding AmoNa to exert its anti-*H. pylori* effect. Batch rebinding test demonstrated a preferential rebinding effect of NQA toward the Dual-MIPs. *In vivo*fluorescence imaging showed the prolonged residence time of Dual-MIPs in *H. pylori* infected mice stomachs after intragastric administration of nanoparticles.*In vivo H. pylori* clearance tests indicated Amo/Dual-MIPs had a better aniti-*H. pylori* effect than amoxicillin powder did. In conclusion, Amo/Dual-MIPs may provide an alternative drug delivery strategy for anti-*H. pylori* therapy.

1. Introduction

Helicobacter pylori infection was the main factor causing chronic gastritis, gastric ulcer, *etc.* (Li et al., 2014; Ertem, 2013; Kim et al., 2008). In recent years, *H. pylori* treatment regimens were mainly based on triple or quadruple therapy, but the extensive application of anti-*H. pylori* antibiotic treatment contributed to ever-increasing rate of treatment failure (Graham and Fischbach, 2010). The possible reason leading to this phenomenon is mainly because *H. pylori* parasitize in parietal cell gaps covered by highly viscoelastic mucus layer. The mucus layer hinders drug molecules to penetrate and quickly reach the site where bacteria parasitized (Cone, 2009; Shah et al., 1999). In addition, gastric mucus regenerates rapidly, making antibiotics hardly achieve sufficient contact with bacteria. The only solution was to increase the clinical dose and administration frequency which unfortunately always led to poor patient compliance and increasing risk of bacterial resistance. In order to reduce the side effects of antibiotics while improving the therapeutic effect, new therapies need to be developed urgently (Zullo et al., 2013; Kuo et al., 2012; Wren et al., 2014). Among all the attempts, antibiotics loaded mucoadhesive drug delivery systems (MDDS) were widely researched (Liu et al., 2005; Zambito et al., 2013; Albertini et al., 2009). All the studies were to prolong the retention time of the antibiotics near *H. pylori* infected sites by interactions between the MDDS and gastric mucin through van der Waals, hydrogen bonds, *etc.* However, this non-specific adhesion effect is always affected due to varying turnover time and composition of mucus, different behavior of mucoadhesive polymers over the pH range and physiological or disease conditions, which limits the application of this technique (Chiou et al., 2009; Chowdary and Rao, 2004).

Lpp20, a conservative outer membrane lipoprotein, is expressed in all *H. pylori* strains. Studies indicated that it was the potential drug target of developing *H. pylori* vaccines and new therapeutic strategies. There exists an exposing antigen fragment of

Abbreviations: H. pylori, *Helicobacter pylori*; Lpp20, a conservative outer membrane lipoprotein expressed in all *H. pylori* strains; NQA, a fraction of Lpp20 (N-terminal amino acid sequence 83-115; MNQA, myristic acid modified NQA; AmoNa, amoxicillin sodium; MNQA-MIPs, MNQA imprinted nanoparticles; Amo-MIPs, AmoNa imprinted nanoparticles; Dual-MIPs, both MNQA and AmoNa imprinted nanoparticles; Amo/Dual-MIPs, amoxicillin loaded Dual-MIPs; NIPs, none imprinted nanoparticles.

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Lpp20 known as NQA (N-terminal amino acid sequence 83-115). The molecular weight of NQA is about 3585 and its isoelectric point is 9.52 (Kosterzynska et al., 1994; Keenan et al., 2000).

The molecular imprinting process involves the synthesis of pre-polymer in the presence of template molecule and functional monomer by covalent, non-covalent or metal synergy. Then the polymerization of the functional monomers is initiated and the template molecules are immobilized in the polymer. Finally, the template molecules are washed, leaving cavities inside or on the surface of the polymer. The cavity left in the polymer is complementary to the template size, shape and functional group. Specific binding will occur when the polymer materials encounter the template molecules again (Janiak and Kofinas, 2007). According to Zeng's work and our previous studies (Zeng et al., 2010; Sun et al., 2011), "surface imprinting" was more likely to be achieved when the template molecules were amphiphilic during an inverse microemulsion polymerization process. Under this consideration, NQA was modified with myristic acid (MNQA) and used as the template to imprint the surface capture sites for the entire Lpp20, thus targeting *H. pylori* by means of an "epitope imprinting" strategy (Nishino et al., 2006; Xue et al., 2009; Pan et al., 2009). Besides, in a bid to improve drug payload and release properties, we made attempt to employ amoxicillin sodium (AmoNa) as a second template to prepare both AmoNa and MNQA imprinted nanoparticles (Dual-MIPs). Batch rebinding test was applied to evaluate the rebinding effect of NQA on Dual-MIPs. *In vivo* and *ex vivo*fluorescence imaging was designed to investigate the gastric retention effect of Dual-MIPs. Finally, AmoNa was reloaded to the nanoparticles (Amo/Dual-MIPs) by incubating the Dual-MIPs with AmoNa solutions for a period of time. *In vitro* drug release performance was studied. A preliminary *in vivo*experiment was carried out to evaluate the anti-*H. pylori* effect of Amo/Dual-MIPs.

2. Materials and methods

2.1. Materials

Acrylamide, *N*,*N*′-methylene bisacrylamide (BisAM), ammonium persulfate (APS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were purchased from Aladdin Reagent. Dioctyl sulfosuccinate, sodium salt (AOT), Brij 30 were supplied from J&K Chemicals. NQA (MW 3585.02 Da, pI 9.5, purity 98.6%), myristic acid modified NQA (MNQA, MW 3795.38 Da, purity 98.6%) were obtained from GL Biochem. Amoxicillin Sodium (AmoNa) was purchased from Meilun Bio (Dalian, China). Near-infrared fluorescence IR-783 (2-[2-[2-chloro-3-[2-[1,3-dihydro-3,3-dimethyl-1-(4-sulfobutyl)-2H-indol-2-ylidene]-ethylidene]-1-cyclohexen-1-yl]-ethenyl]-3) was obtained from Sigma. All other chemicals and solvents were analytical grade and used as received. Deionized water was produced by a MilliQ water system from Millipore (Merck). *H. pylori* strain SS1 was obtained from Institute of Gastrointestinal Disease, Yan Chai Hospital. C57BL/6 mice (SPF level, 20.0 ± 2.0 g) were purchased from the Department of Laboratory Animal Science, Fudan University.

2.2. Preparation of nanoparticles via inverse microemulsion polymerization

Dual templates (MNQA and AmoNa) imprinted nanoparticles (Dual-MIPs) were prepared as reported with some modifications (Zeng et al., 2010). Briefly, acrylamide (0.45 g), BisAM (0.13 g), MNQA (3 mg) and AmoNa (150 mg) were dissolved in 1.0 mL of water to obtain a solution of about 1.5 mL. Then 1.0 mL of the solution was withdrawn and added dropwise into deoxygenated solution of hexane (22.0 mL) with surfactants AOT (0.80 g) and Brij 30 (1.54 g). The solutions were stirred for 1 h to form the microemulsion. The polymerization was initiated at room temperature upon addition of APS (100 μL, 10% (w/v)) and TEMED (50 μL). The microemulsion gradually appeared opalesque during polymerization over 2 h. The nanoparticles were then precipitated and washed 5 times with excess ethanol to remove the unreacted monomers, templates and surfactants. The nanoparticles were collected and redispersed in water and purified via gel chromatography (G50) to make sure the templates were completely removed. Upon completion, the nanoparticles were lyophilized and kept at room temperature for further characterization.

MNQA-MIPs, Amo-MIPs and NIPs were prepared under identical conditions in the absence of one or both templates.

Near-infrared fluorescence probe IR-783 encapsulated Dual-MIPs and NIPs were prepared for *in vivo* imaging test. Briefly, 10 mg of IR-783 was added into 1 mL of the aqueous phase prior to emulsification and the reaction system was then treated exactly as the same preparation procedures of imprinted nanoparticles. The size and fluorescence intensity of (IR-783)/Dual-MIPs and (IR-783)/NIPs were determined (Zhang et al., 2015).

2.3. Characterization of the nanoparticles

The hydrodynamic diameter and zeta potential of the nanoparticles were measured using a Malvern Zetasizer® Nano ZS (Malvern, Worcestershire, UK). The nanoparticles were dispersed in deionized water and sonicated prior to measurements.

The morphology of Dual-MIPs was observed by transmission electron microscopy (TEM) (JEM-2100F, JEOL, Japan). A drop of Dual-MIPs suspension was added onto the carbon-coated grid and air-dried, following negative staining with 2% phosphotungstic acid. Then the sample was examined directly.

The fluorescence intensity of IR-783 encapsulated Dual-MIPs and NIPs were determined by IVIS spectrum (Xeogen, USA).

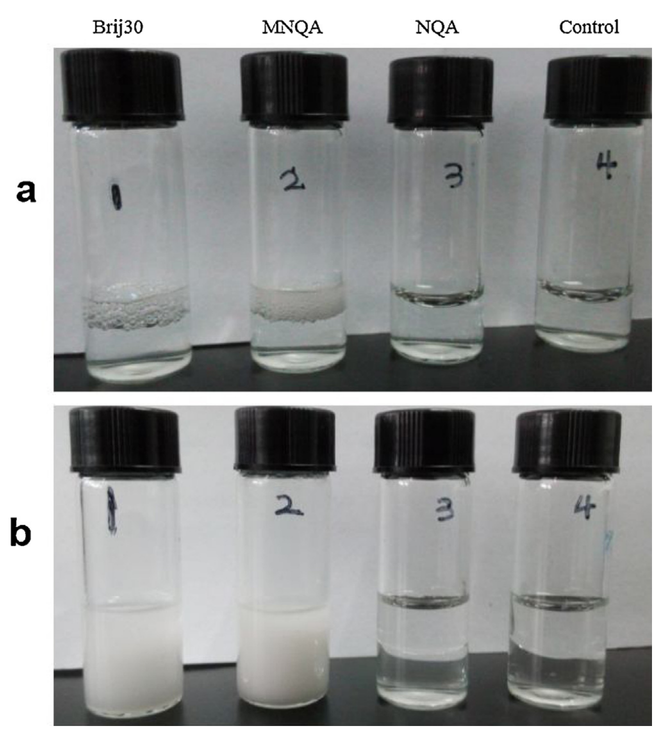

Fig. 1. Surfactant-like effect of MNQA in different solvents: (a) 1 mL of water in addition with 1 mg of Brij 30 (vial1), 1 mg of MNQA (vial 2), 1 mg of NQA (vial 3) and none (control) (vial 4). (b) Mixed solvent (1 mL of water and 1 mL of hexane) in addition with 1 mg of Brij 30 (vial 1), 1 mg of MNQA (vial 2), 1 mg of NQA (vial 3) and none (control) (vial 4).