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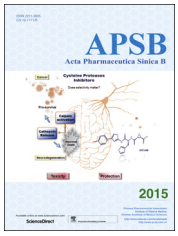

ORIGINAL ARTICLE

Preliminary investigations into surface molecularly imprinted nanoparticles for *Helicobacter pylori* eradication

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Abstract This paper reports investigations into the preparation and characterization of surface molecularly imprinted nanoparticles (SMINs) designed to adhere to *Helicobacter pylori (H. pylori)*. Imprinted nanoparticles were prepared by the inverse microemulsion polymerization method. A fraction of Lpp20, an outer membrane protein of *H. pylori* known as NQA, was chosen as template and modified with myristic acid to facilitate its localization on the surface of the nanoparticles. The interaction between these SMINs with the template NQA were evaluated using surface plasmon resonance (SPR), change in zeta potential and fluorescence polarization (FP). The results were highly consistent in demonstrating a preferential recognition of the template NQA for SMINs compared with the control nanoparticles. *In vitro* experiments also indicate that such SMINs are able to adhere to *H. pylori* and may be useful for *H. pylori* eradication.

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1. Introduction

During recent decades, mucoadhesive drug delivery systems (MDDS) for *Helicobacter pylori* (*H. pylori*) eradication have been frequently reported. The ability of MDDS to adhere to the mucus layer prolongs their retention time in the gastrointestinal tract and thereby enables the loaded drug to interact with *H. pylori* for a longer time. However, this non-specific adhesion is affected not only by varying turnover time and composition of mucus but also by how the mucoadhesive polymer used to make MDDS responds to changes in pH and physiological or disease conditions. Such variability seriously limits the application of this technique[1]. This paper reports the results of an investigation into the preparation and characterization of surface molecularly imprinted nanoparticles (SMINs) designed to bind to *H. pylori*.

Molecular imprinting involves the synthesis of polymers in the presence of a template molecule. The process produces polymers with surface binding sites that complement those of the template in terms of its size, shape and functional group orientation[2]. Molecularly imprinted polymers (MIPs) have been widely recognized as promising alternatives to enzymes, antibodies and natural receptors due to their specificity, robustness and reusability[3]. However, most of the successful examples are imprinted with low molecular weight compounds while imprinting with larger molecules such as peptides and proteins, or with complex systems such as viruses and cells is still in its infancy. According to Kryscio et al.[4], it is now evident that the general design principles of low molecular weight MIPs do not apply to the macromolecular regime. The monomers commonly employed in MIPs significantly alter the template conformation prior to polymerization and compromise the specific recognition of the template.

Three main approaches have been developed for protein imprinting[2]: (1) Bulk imprinting where the protein template is wholly imprinted in the bulk of the polymer matrix and is bound as a whole molecule by functional monomers; (2) surface imprinting where the protein template is partially imprinted in the bulk of the polymer matrix and binding sites are at or close to the surface; and (3) epitope imprinting where only a small part of the protein is imprinted but the resulting MIPs are able to recognize the whole protein. In bulk imprinting, the binding sites of the template proteins tend to be restricted within the polymer matrix and are only exposed by grinding the matrix. As a result, surface and epitope imprinting have attracted increasing interest.

To mimic the genuine "antigen-antibody" interaction, it is important that well-designed imprints are evenly distributed on the surface of SMINs. To achieve this goal, Zeng et al.[5] initiated a so-called "general strategy for surface imprinting" in which a hydrophilic peptide could be located at the surface of nanoparticles after modification. We have used this approach in preparing SMINs.

Lpp20 is a conserved 20 kDa outer membrane lipoprotein antigen specifically expressed by all strains of *H. pylori* that has been shown to act as a potential target for vaccine and pharmacotherapy[6,7]. In our laboratory, an exposed antigenic domain of Lpp20 (amino acids 83–115 from the N-terminus, abbreviated as NQA) was imprinted on the surface of nanoparticles. According to research by our group[8] and Zeng et al.[5], "surface imprinting" is more likely to be achieved during an inverse mini-emulsion polymerization process when the template molecules are amphiphilic. Based on this consideration, the hydrophilic NQA was modified with a long hydrocarbon tail (from myristic acid (Myr)) and used as the template to imprint surface capture sites for the entire Lpp20 lipoprotein. Such an epitope imprinting strategy has been previously used to capture *Staphylococus aureus*[9–11].

Some reports have argued that one of the technical challenges in molecular imprinting of proteins relates to the quantitation of the resulting binding. In many cases, determination of the extent of binding is unreliable and leads to unconvincing results[12]. Therefore, newly designed approaches to quantitation are required. In the current study, several approaches to assess protein–protein interactions or the bioadhesive properties of polymers including surface plasmon resonance (SPR), fluorescence polarization (FP) and changes in zeta potential were applied to evaluate the binding of the template to the MIPs. Preliminary *in vitro* studies were also performed to evaluate the efficacy of the MIPs to specifically adhere to *H. pylori*. Further studies on therapeutic drug delivery by means of this type of SMINs are underway.

2. Materials and methods

2.1. Materials

Acrylamide, *N*,*N*′-methylenebisacrylamide (BisAM), ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were purchased from Aladdin Reagents Co., Ltd. (Shanghai). 5-Carboxyfluoresccein (FAM) and dioctyl sulfosuccinate sodium salt (AOT) were purchased from Sigma-Aldrich. Brij 30 was supplied by J&K Chemicals. NQA (MW 3582 Da, pI 9.5), myristic acid modified NQA (Myr-NQA), and FAM modified NQA (FAM-NQA) were obtained from GL Biochem. The Biacore sensor chip CM5 and HBS-EP buffer were purchased from GE Healthcare. All other chemicals and solvents were analytical grade and used as received. Deionized water was produced by a Milli-Q integral water purification system (Millipore).

2.2. Cell lines and H. pylori strain

Human gastric epithelial (AGS) cells were obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences. Cells were maintained in Ham's F-12 medium (GIBCO BRL, Grand Island, NY, USA) containing 10% fetal calf serum and cultured in incubators maintaining 5% CO_2 under microaerophilic conditions at 37 °C. *H. pylori* strain SS1 was obtained from the Institute of Gastrointestinal Disease, Yan Chai Hospital.

2.3. Preparation of nanoparticles

Myr-NQA imprinted nanoparticles (Myr-NQA-MINs) were prepared as previously reported with some modifications[5]. Briefly, a monomer solution was formed by dissolving acrylamide (0.45 g) and BisAM (0.13 g) in 1.0 mL water. Myr-NQA (8.0 mg) was added to the solution and magnetically stirred to facilitate effective monomer-template interaction. Subsequently, 1.0 mL of this monomer-template solution was added dropwise to a deoxygenated mixture of hexane (21.5 mL), AOT (0.70 g) and Brij 30 (1.50 g), and stirred continuously for 30 min. Finally, APS solution (10% *w/v*, 50 µL) followed by TEMED (25 µL) were added to the mixture to initiate polymerization. The reaction was allowed to proceed at room temperature for 2 h after which nanoparticles were precipitated and washed 4 times with excess ethanol to remove unreacted monomer, template and surfactant.