International Journal of Pharmaceutics 502 (2016) 232–241

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Novel polymeric nanoparticles targeting the lipopolysaccharides of *Pseudomonas aeruginosa*

Y. Long[a], Z. Li[a], Q. Bi[a], C. Deng[a], Z. Chen[a], S. Bhattachayya[b,**], C. Li[a,*]

[a] College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, PR China
[b] WPI-AIMR, Tohoku University, Japan

ARTICLE INFO

Article history:
Received 17 October 2015
Received in revised form 29 January 2016
Accepted 14 February 2016
Available online 17 February 2016

Keywords:
Polymeric nanoparticles
Molecular imprinting
Lipopolysaccharide
Drug targeted delivery
Photodynamic therapy

ABSTRACT

Considering outburst of various infectious diseases globally, nanoparticle assisted targeted drug delivery has emerged as a promising strategy that can enhance the therapeutic efficacy and minimize the undesirable side effects of an antimicrobial agents. Molecular imprinting is a newly developed strategy that can synthesize a drug carrier with highly stable ligand-like 'cavity', may serve as a new platform of ligand-free targeted drug delivery systems. In this study, we use the amphiphilic lipopolysaccharides, derived from *Pseudomonas aeruginosa* as imprinting template and obtained an evenly distributed sub-40 nm polymeric nanoparticles by using inverse emulsion method. These molecularly imprinted nanoparticles (MIPNPs) showed specific binding to the lipopolysaccharide as determined by fluorescence polarization and microscale thermophoresis. MIPNPs showed selective recognition of target bacteria as detected by flow cytometry. Additionally, MIPNPs exhibited the *in vivo* targeting capabilities in both the keratitis model and meningitis model. Moreover, the photosensitizer methylene blue-loaded MIPNPs presented significantly strong inhibition of bacterial Growth, compared to non-imprinted controls for *in vitro* model of the photodynamic therapy. Our study shows an attempt to design a magic bullet by molecular imprinting that may provide a novel approach to generate synthetic carrier for targeting pathogen and treatment for a variety of infectious human diseases.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The escalating challenge of controlling infection rates has necessitated the development of novel pathogen detection and treatment methods. Cell-specific recognition combining receptor-ligand interactions with nanotechnology has become an important tool in advancing such methods. Nanoscale modification of a probes or agents with a targeting-moiety towards a cell surface biomarker can be used in clinical applications to effectively target cells (Hofmann et al., 2012; Li et al., 1999). For example, a designed peptide ligand of the *Staphylococcus epidermidis* surface protein, SdrG, was successfully implemented as a specific targeting motif for the delivery of nanogold particles and antibiotics that target bacteria (Davis et al., 2001). However, widely used targeting agents, such as antibodies, peptides, and aptamers, suffer from limitations including instability in physiological environments and relatively high production costs (Nishino et al., 2006). Novel strategies to design therapeutically viable ligands are required to address this challenge.

Molecular imprinting, a relatively new technique by which cavities are shaped into polymer matrices based on template molecules, has been advanced in recent years. Following these advances, small molecule recognition is now successfully applied in several marketed products used for separation, purification, and detection. The field continues to make remarkable progress forward in the molecular imprinting of bio-macromolecules (Cutivet et al., 2009; Li et al., 2013; Pan et al., 2009; Shinde et al., 2012), and the methods developed could serve as novel routes for enabling simpler biomolecular recognition (Akiyama et al., 2001; Pan et al., 2013; Svenson et al., 2004; Vlatakis et al., 1993). For example, the "plastic antibody", developed by the Shea research group, effectively binds and neutralizes to venomous peptides *in vivo*, thereby providing a promising technology for use in diagnosis and therapy (Hoshino et al., 2010). Additionally, this group created a general approach for preparing synthetic polymer nanoparticles (NPs) with surface binding sites for hydrophilic bio-molecules. Specifically, the synthetic polymeric NPs are produced

* Corresponding author at: College of Pharmaceutical Sciences, Southwest University, No. 2, Tiansheng Road, Beibei District, Chongqing 400715, PR China.
** Corresponding author.
E-mail addresses: bhattacharyya.sanjib2k12@gmail.com (S. Bhattachayya), chongli2009@gmail.com (C. Li).

http://dx.doi.org/10.1016/j.ijpharm.2016.02.021
0378-5173/© 2016 Elsevier B.V. All rights reserved.

by inverse microemulsion polymerization, where a modified amphiphilic peptides are embedded onto the NP surface and then dialyzed to remove the template molecule, thereby creating accessible binding sites with strong affinities for that targeting peptide molecule (Zeng et al., 2009). This technique provides a solid foundation for the design of nanocarriers with molecularly imprinted 'cavities' as highly stable binding sites that can specifically recognize the targets, thus valuable for targets therapy to any diseases.

Pseudomonas aeruginosa is a major opportunistic human pathogen. It frequently causes hospital-acquired infections that have the highest fatality rate among all gram-negative bacteria (Wisplinghoff et al., 2004). *Pseudomonas* can infect any part of the body, including external body tissues and internal organs. For example, *P. aeruginosa* can infect the brain and spinal cord and lead to meningitis, brain abscesses, and potentially death (Huang et al., 2007). It can also invade the ocular region, following an injury, and ultimately cause ulcers of the cornea (keratitis), which can lead to sight-threatening syndromes with compromised quality of life (Tajima et al., 2014). Thus, it is essential to develop novel methods to combat this dangerous pathogen. Lipopolysaccharide (LPS) is the principal structural component of the outer membrane of all gram-negative bacteria including *P. aeruginosa*. LPS levels are usually positively correlated with disease severity, and LPS confers bacterial serotype specificity that can be applied in pathogen identification (Borrelli et al., 1999; Kondakova et al., 2003; Shelburne et al., 1993). The exposed hydrophilic domain of LPS can also provide a valuable target in the capture of whole bacteria for biomedical applications (Pengsuk et al., 2013). Because of the amphiphilic nature of LPS with its highly hydrophilic saccharide component, it is possible to use LPS as a natural template to fabricate a synthetic nanoparticle *via* inverse microemulsion polymerization. Here, we report the preparation, characterization and functional validation of a molecularly imprinted nanocarrier that exhibited strong affinity and targeting specificity to *P. aeruginosa* (Scheme 1). We imprinted a molecular cavity or motif specific to LPS on a spherical polymeric NP and we used that cavity bearing polymeric NP as a specific targeting to the *P. aeruginosa* mediated infection. We demonstrated the targeting efficacy of MIPNPs in two different animal models, the keratitis model and meningitis model, where the imprinted NP selectively accumulated the site of infection. We also report the *in vitro* therapeutic efficacy of the MIPNPs by using photodynamic therapy (PDT).

2. Materials and methods

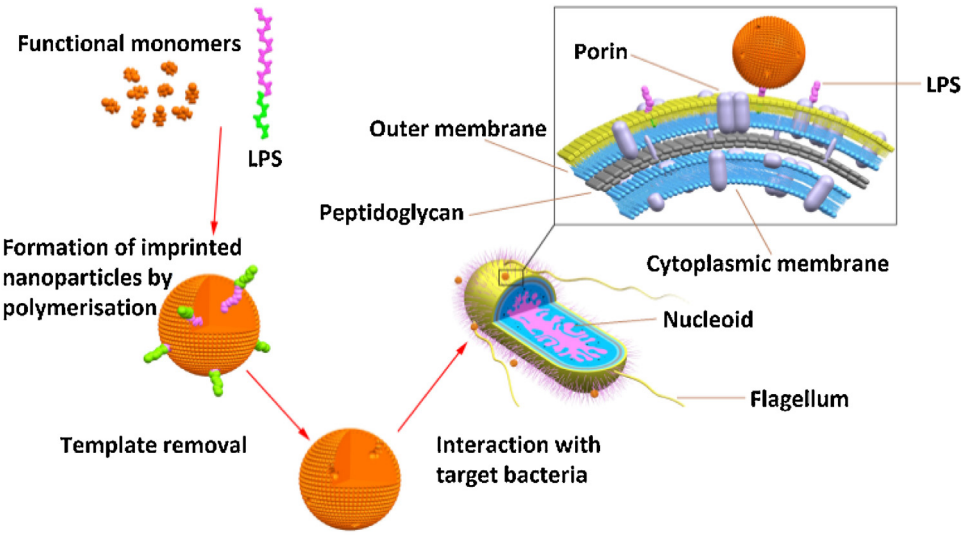
2.1. Materials

The following materials were obtained from commercial providers: acrylamide (AAM), N,N′-methylenebisacrylamide (BIS), ammonium persulphate (APS), polyethylene glycol dodecyl ether, (Brij-30), and fluorescein isothiocyanate (FITC) were from Aladdin (Shanghai, China); lipopolysaccharides from *P. aeruginosa* 10 (LPS), dioctyl sulfosuccinate sodium salt, 96% (AOT), N,N,N′,N′-tetramethyl ethylenediamine (TEMED), doxorubicin hydrochloride (DOX), and fluoresceinamine isomer II (FAM) were from Sigma Aldrich (St. Louis, MO, USA); methylene blue (MB) was from Kelong Chemical Co., Ltd. (Beijing, China); 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 (IR-783) was from Fanbo Biochemicals Co., Ltd. (Beijing, China). All the chemicals used in this study were analytical grade.

Kunming mice (18–20 g) and Albino New Zealand rabbits (2.0–2.5 kg) were obtained from the Experimental Animal Center of Third Military Medical University (Chongqing, China), and kept under specific pathogen free (SPF) conditions. All animal experiments were performed in accordance with guidelines approved by the ethics committee of the College of Pharmaceutical Sciences, Southwest University, Chongqing, PR China.

2.2. Preparation of nanoparticles

The LPS molecularly imprinted nanoparticles (MIPNPs) were synthesized as follows: AAM (35.5 mg) and BIS (11.5 mg) were dissolved in 100 μL distilled water. LPS (0.8 mg) was added to the mixture and stirred for about 30 min to obtain the pre-polymer. AOT (80 mg) and Brij 30 (154 mg) dissolved in 4 mL hexane were added to the mixture under a nitrogen atmosphere. The reaction was allowed to continue for 1 h. Subsequently, APS (5 μL of 10% (w/v)) and TEMED (2.5 μL) were added to the solution and stirred for 2 h. This entire procedure was carried out at room temperature. Finally, ethanol was added to the polymerized solutions to precipitate the nanoparticles before they underwent centrifugation at 4500 rpm for 10 min. This process was repeated three times to remove LPS, surfactants, and unreacted monomers (Hoshino et al., 2008; Zeng et al., 2009). Non-imprinted nanoparticles



Scheme 1. Molecularly imprinted nanoparticles for specific recognition of bacteria.

Download English Version:

https://daneshyari.com/en/article/2501067

Download Persian Version:

https://daneshyari.com/article/2501067

Daneshyari.com