Biomaterials xxx (2013) 1–10

Contents lists available at ScienceDirect

Biomaterials

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

Nitric oxide integrated polyethylenimine-based tri-block copolymer for efficient antibacterial activity

Junghong Park [a,b], Jihoon Kim [a,b], Kaushik Singha [a,b], Dong-Keun Han [c], Hansoo Park [d,**], Won Jong Kim [a,b,*]

[a] Center for Self-assembly and Complexity, Institute for Basic Science, Pohang 790-784, Republic of Korea
[b] Department of Chemistry, Polymer Research Institute, Pohang University of Science and Technology (POSTECH), Pohang 790-784, Republic of Korea
[c] Biomaterials Research Center, Korea Institute of Science and Technology, Seoul 130-650, Republic of Korea
[d] School of Integrative Engineering, 84 Heukseok-Ro, Dongjak-gu, Chung-Ang University, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 24 June 2013
Accepted 19 July 2013
Available online xxx

Keywords:
N-Diazeniumdiolates
Nitric oxide delivery
Antibacterial activity
Methicillin-resistance

ABSTRACT

The work demonstrated a successful synthesis of nitric oxide (NO)-releasing material and its antibacterial effect on Gram-negative *Escherichia coli* (*E. coli*), Gram-positive *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *S. aureus* (MRSA). The polymeric support composed of thermosensitive Pluronic F68 having good biocompatibility and branched polyethylenimine (BPEI) housed N-diazeniumdiolates (NONOates) which could store and release NO under appropriate physiological condition. The developed F68–BPEI–NONOates releases a sufficient amount of NO under physiological condition to elicit effective killing of *E. coli*, *S. aureus* and MRSA. The antibacterial ability of the released NO was compared to untreated control or unmodified F68 polymer by using confocal microscopy; F68–BPEI–NONOates demonstrated excellent antibacterial activity with *in vitro* low cytotoxicity. TEM investigation also revealed the destruction of bacteria membrane caused by NO. The effectiveness of F68–BPEI–NONOates against resistant strains such as MRSA provides a very simple but highly efficient strategy to combat drug-resistant bacterial infections.

1. Introduction

Diseases arising from bacterial infections remain a serious concern and under community and nosocomial settings pose a challenge to treat the patients particularly afflicted with antibiotic resistance bacterial strains. Among antibiotic resistance bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) infections contracted in nosocomial settings inflict high morbidity and mortality worldwide and the number of victims has been increasing continuously [1]. Unfortunately, resistance to methicillin confers resistance to all penicillin and cephalosporin. The available therapeutic choices for severe MRSA infections are restricted to a few antibiotics such as vancomycin. Apart from the impediments associated with acquired resistance and side effects, delivery efficiency also imposes considerable problems in achieving high therapeutic outcome. So far, many strategies have been adopted for delivery of antibiotic agents. Sande et al. [2] reported that vancomycin-encapsulated liposome showed a good killing ability compared to free vancomycin in a murine infection model. However, associated side effects such as diarrhea [3], vomiting [4], gastroenteric trouble [5] spur incessant quest for more efficient therapeutic agents and effective delivery strategies. For instance, Mahmoudi et al. [6] reported that silver-coated SPION showed a good antibacterial activity against resistant bacteria. As enhanced therapeutic effect is intimately related with the efficient delivery of therapeutic pay load i.e., antibiotics, considerable efforts have been devoted to develop various advanced delivery strategies involving silver coating [6–8], silver nanoparticle [9–11], polymer and peptide coating [12–16]. Recently, nitric oxide (NO), a reactive radical species with broad spectrum antibacterial activity has attracted immense interest for their bactericidal activity especially against resistant bacteria [17,18]. In addition to NO, the other active species which are generated through the interaction between NO and reactive oxygen intermediates, have been considered to play crucial role in imparting antibacterial activity. Such active species may include NO radical (NO•), nitrogen dioxide (NO_2•), dinitrogen trioxide (N_2O_3) and peroxynitrite ($ONOO^-$) [19,20]. These kinds of

* Corresponding author. Center for Self-assembly and Complexity, Institute for Basic Science, Pohang 790-784, Republic of Korea. Tel.: +82 54 279 2104; fax: +82 54 279 3399.
** Corresponding author. Tel.: +82 2 820 5804; fax: +82 2 814 2651.
E-mail address: wjkim@postech.ac.kr (W.J. Kim).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.07.064

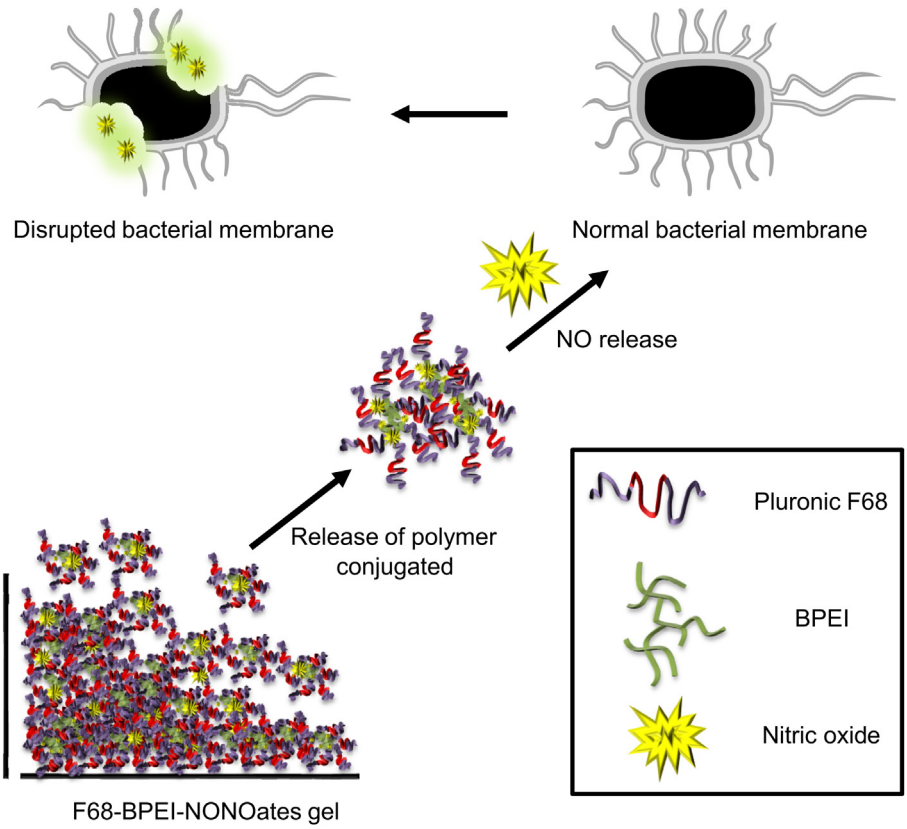
oxidants have demonstrated their ability to trigger deamination of DNA [21], causing strand break [22], and to induce lipid peroxidation [23]. Therefore, many researchers have developed exogenous NO delivery tools via NO donors such as S-nitrosothiol (RSNO) [24–26], NO aspirin [27], nitroglycerin [28] and N-diazeniumdiolate (NONOates) [29–32] which can store and release NO at opportune moments. Interestingly, macromolecular-based NO delivery system is known to display better antibacterial properties compared to that elicited by small molecule derived NO [30]. Stasko et al. [24] synthesized S-nitrosothiol-modified dendrimers which could release NO. Similarly, Riccio et al. [26] reported that S-nitrosothiol-modified xerogels generated NO under broad-spectrum light. NONOates was used prevalently to deliver exogenous NO due to the facile synthetic procedure involving simple treatment of secondary amines with NO gas at 80 psi. In addition, proton-initiated instantaneous release of 2 mol of NO from one mol of NONOates under physiological conditions makes it an efficient scaffold for NO delivery [32]. Previously, we reported a novel NO-releasing polymeric system, developed by conjugating NONOates into branched polyethylenimine (BPEI), which was linked with thermosensitive Pluronic polymer. The reported hydrogel-type NO-releasing system showed enhanced proliferation of endothelial cells, and inhibition of smooth muscle cell growth [31]. Based on this result, we assume that large amount of NO could be loaded into this polymer system and a controlled release of NO could be attained. This study illustrates the modification and optimization of this NO delivery system, along with its application as antibacterial agent (Scheme 1).

We used Pluronic F68, a thermosensitive and water soluble triblock copolymer with composition $(PEO)_{78}$–$(PPO)_{30}$–$(PEO)_{78}$ [33] which can act as a non-ionic surfactant and inhibit platelet aggregation [34]. It has been approved by Food and Drug Administration (FDA) [35,36], and commonly used to support changeable surface as a scaffold with no cytotoxicity for cells. The hydrophobic portion of this tri-block copolymer can also be utilized to encapsulate the hydrophobic drugs [37]. F68 forms sol at low temperature but turns into gel at body temperature [38,39], making itself a good substrate for injectable hydrogel. NO-loaded F68 polymer can act as depot to store and release NO. It can be expected that low concentration of NO-conjugated F68 will be released from the surface of depot in sustained manner, and then show antibacterial activity. BPEI (M_w 1800 Da), containing primary (25%), tertiary (25%), and secondary amines (50%) [40], possesses strong positive charge in physiological condition and has been used for conjugating NONOates under 80 psi of NO gas [31]. We demonstrated the characterization and investigated the antibiotic activity of F68–BPEI–NONOates as an antibacterial agent with low cytotoxicity.

2. Materials and methods

2.1. Materials

Pluronic F68 ($(PEO)_{78}$–$(PPO)_{30}$–$(PEO)_{78}$) *p*-nitrophenyl chloroformate (*p*-NPC), tetrahydrofuran, potassium bromide, dichloromethane (DCM), diethyl ether, dimethyl sulfoxide and methanol were purchased from Sigma Aldrich (St. Louis, MO). Sodium methoxide (NaOMe) was purchased from Acros Organics (Geel, Belgium). Triethylamine (TEA) was obtained from Samchun Chemicals (Pyeongtaek, Korea). Branched PEI (BPEI, M_w 1.8 kDa) was obtained from Polysciences, Inc., (Warrington, PA). The dialysis membrane was purchased from Spectrum Laboratories (Rancho Dominguez, CA). NO gas was purchased from HANA gas (Gimhae, Korea). Argon (Ar) gas was purchased from BOC gas (Pohang, Korea). Tryptic soy broth (TSB), LB broth, tryptic soy agar (TSA) and nutrient agar were obtained from Difco™ (Franklin Lakes, USA). *Escherichia coli* (*E. coli*; 25922), *S. aureus* (*S. aureus*; 29213) and methicillin-resistant *S. aureus* (MRSA; 33591) were purchased from Korean Culture Center of Microorganisms (Seoul, Korea). Dulbecco's phosphate buffered saline (DPBS) and Live/Dead® Baclight™ Bacterial Viability Kits (L7012) were obtained from Invitrogen (Molecular Probes Inc., Eugene, Oregon).



Scheme 1. Schematic illustration for F68–BPEI–NONOates-mediated antibacterial activity.

Please cite this article in press as: Park J, et al., Nitric oxide integrated polyethylenimine-based tri-block copolymer for efficient antibacterial activity, Biomaterials (2013), http://dx.doi.org/10.1016/j.biomaterials.2013.07.064