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Supramolecular nanofibers self-assembled from cationic small molecules derived from repurposed poly(ethylene teraphthalate) for antibiotic delivery

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Abstract

Low molecular weight cationic compounds were synthesized from re-purposed poly(ethylene teraphthalate) (PET) and used to self-assemble into high aspect ratio supramolecular nanofibers for encapsulation and delivery of anionic antibiotics. The antibiotic piperacillin/tazobactam (PT) was successfully loaded into the nanofibers through ionic interaction between anionic PT and the cationic nanofibers without loss of the nanofiber features. These PT-loaded nanofibers demonstrated high loading efficiency and sustained delivery for PT. The antimicrobial activity of PT-loaded nanofibers remained potent towards both Gram-positive and Gram-negative bacteria. Importantly, in a *P. aeruginosa*-infected mouse skin wound model, the treatment with the PT-loaded nanofibers was more effective than free PT for wound healing as evidenced by the significantly lower *P. aeruginosa* counts at the wound sites and histological analysis. This strategy can be applied to deliver a variety of anionic antibiotics for improved treatment efficacy of various infections.

Key words: Cationic small molecules; Supramolecular assembly; Nanofibers; Antibiotic delivery; Wound infection

Faced with the emergence of antibiotics-resistance bacteria, the treatment of bacterial infections with clinical antibiotics is an increasingly daunting task. One of the factors driving the persistence of antibiotics-resistant bacteria is exposure to non-optimal antibiotics concentration during the course of treatment.[1] While it is putative that exposure to high concentrations of antibiotics drives the selection for resistant strains, studies have also maintained that exposure to sub-inhibitory concentrations is an important selection factor.[1] A recent report revealed that exposure of vancomycin-susceptible MRSA to sub-inhibitory concentrations of beta-lactam antibiotics developed vancomycin-resistance in MRSA.[2] High doses are needed to treat drug-resistant infections. On the other hand, antibiotics instability, premature elimination and metabolism could result in a sub-inhibitory concentration in infection sites.[3] Therefore, increasing therapeutic concentration of antibiotics in the infection sites also requires high doses. However, exposure to high concentrations of antibiotics may lead to undesirable systemic side-effects.[4] To maximize efficacy and mitigate drug-resistance development, it is imperative to deliver antibiotics in a sustained manner so that effective concentrations can be achieved for a prolonged period of time at the infection sites.

Use of nanocarriers for antibiotics delivery has demonstrated much potential in shifting the paradigm for the treatment of bacterial infections.[4–6] Particularly, nanoparticulate drug delivery systems provide an avenue to achieve the controlled and sustained release of antibiotics at therapeutic concentrations. Likewise, they offer a convenient platform for co-delivery of combination antibiotics.[3,4] Nanocarriers could also extend drug half-life, improve therapeutic index, optimize the pharmacokinetics profiles, and consequently decrease the frequency of drug administration and encourage patient compliance.[3,4] At present, a variety of nanocarriers, comprising of liposomes, polymeric nanoparticles, dendrimers and solid liquid nanoparticles *etc.*, are investigated for drug delivery.[3] However, shortcomings noted in some of these

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nanocarriers include low stability of lipid-based carriers, short drug expulsion duration and low drug loading capacity in solid liquid nanoparticles.[7] Polymeric nanocarriers have been deemed to be one of the most promising delivery systems that confer a number of advantages such as biocompatibility, biodegradability, mechanical stability in biological environment and negligible immunogenicity.[4] Moreover, the physio-chemical properties (*i.e.* size, functional group, charge) of polymer nanocarriers can be tailored precisely at a molecular level to enhance drug loading and control drug release.[4,8] Affirmative results have been reported, where doxycycline-loaded poly($_{D,L}$-lactide-co-glycolide) and poly(ε-caprolactone) nanoparticles exhibited increased antimicrobial efficacy compared to free antibiotic by the virtue of sustained release from the nanoparticles.[3]

Studies have also suggested that the efficacy of drug delivery can be influenced by the size and shape of nanocarriers.[9] Our previous studies demonstrated that nanostructure formation increased local concentration of cationic charges and antimicrobial peptide mass, hence enhancing antimicrobial function.[10] Moreover, the filamentous structure of cylindrical filomicelles demonstrated greater stability in circulation than the spherical micelles by 10-fold, without compromising the rate of uptake by the cells.[11] These preliminary findings illustrate the promise of filamentous nanofibers as drug delivery carriers.

We recently reported that cationic terephthalamide bisurea amphipathic small molecules, derived from selectively depolymerized PET, with exact molecular weights and specific molecular recognition motifs were able to self-assemble into high aspect ratio supramolecular cationic nanofibers. These nanostructures exhibited strong antifungal activity against drug-sensitive and -resistant fungal strains with high selectivity.[12,13] In this study, these cationic nanofibers were used to deliver piperacillin-tazobactam (PT) through electrostatic interaction between the cationic charge in the nanofibers and negative charge in both piperacillin and tazobactam. Piperacillin is a broad-spectrum β-lactam antibiotic, and used in combination with tazobactam (a β-lactamase inhibitor) for the treatment of serious hospital-acquired Gram-negative bacterial infections.[14] Piperacillin-tazobactam is especially active against *P. aeruginosa*. The cationic multivalency of the nanofibers is particularly useful for PT sequestration. These PT-loaded nanofibers exhibited high encapsulation efficiency and sustained delivery, and released PT remained potent towards both Gram-positive and Gram-negative bacteria. In addition, *in vivo* anti-bacterial efficacy of the PT-loaded nanofibers was demonstrated in a *P. aeruginosa*-infected mouse wound model. This is the first example of using small molecules-assembled nanofibers for antibiotic delivery.

Methods

Materials

Tryptic soy broth (TSB) powder was bought from BD Diagnostics (Singapore) and used for broth preparation. *S. aureus* (ATCC No. 29737), Gram-negative bacteria *E. coli* (ATCC No. 13048) and *P. aeruginosa* (ATCC No. 9027) were purchased from ATCC (U.S.A) and re-cultured using the manufacturer's protocols. Piperacillin and tazobactam were provided by Sigma. The synthesis of carbamates, *tert*-butyl (4-aminobutyl)carbamate, and *tert*-butyl 4-(aminomethyl)benzylcarbamate was reported previously.[12] All other chemicals were obtained from Sigma-Aldrich and used as received.

Synthesis

The cationic small molecular compounds were synthesized in three steps (Figure 1),[12] and the synthesis of compound 3a was described as an example. Briefly, an anhydrous dimethylformamide (DMF) solution (16 mL) of pentafluorophenyl carbonate (PFC) (3.97 g, 10.1 mmol) was added to an anhydrous DMF solution (8 mL) of the diamine N^1,N^4-bis(4-aminobenzyl)terephthalamide (4ABTA, **1**) (1.50 g, 4.0 mmol). The reaction mixture was stirred for 1 h at room temperature before the addition of *tert*-butyl (4-aminobutyl)carbamate (2.36 g, 12.6 mmol). The reaction continued overnight under stirring. To remove excess PFC and the diamine, the reaction mixture was precipitated in diethylether (250 mL). The product was then filtered and dried in vacuum (60 °C) to obtain 2a (2.87 g, 90%).

The compound 2a (2.50 g, 3.11 mmol) was then added to trifluoroacetic acid (10 mL) and stirred overnight to form a homogeneous mixture for deprotection. The mixture was then precipitated in diethylether (200 mL), filtered, washed with diethylether several times and dried to yield 3a (2.07 g, 80%). The final product was characterized with ^{1}H NMR (see Supplementary Material). The synthesis details of 3b were described in Supplementary Material.

Zeta potential and critical micelle concentration (CMC) measurements

The measurement of zeta potential and CMC values of the cationic compounds in de-ionized (DI) water was done by Zetasizer 3000 HAS (Malvern Instrument Ltd., Malvern, UK). The detailed protocol is given in the Supplementary Material.

Nanofiber preparation and antibiotics loading

Membrane dialysis method was used to prepare blank nanofibers. The cationic compounds (10 mg) were dissolved in 2 mL DMF and dialyzed against DI water at room temperature (22 °C) for 24 h using a dialysis membrane with a molecular weight cut-off (MWCO) of 1000 (Spectra/Por 7, Spectrum Laboratories Inc.). Piperacillin and tazobactam were dissolved in DI water (mass ratio 8:1[15]) and added to blank nanofibers in a weight ratio of 1:10. The solution was gently stirred at 200 rpm for 3 h and stand for another 3 h at room temperature before characterization. The solution was further purified using a centrifugal filter (MWCO: 2000, Sartorius) to remove free antibiotics. The content of piperacillin was characterized by high performance liquid chromatography (HPLC) (see Supplementary Material).[16] Loading efficiency was calculated using the formula: Final amount of loaded piperacillin/Original amount of piperacillin added.

Transmittance electron microscopy (TEM)

The morphology of the nanofibers was investigated using TEM (FEI Tecnai G^2 F20 electron microscope). Compound 3a and 3b solution (5 μL) was placed on a copper grid coated with carbon film. One min later, 5 μL of phosphotungstic acid 0.1 (w/v) % was added. Filter paper was used to absorb any extra sample on the grid, followed by air-drying at room temperature. The samples were