Antibiofilm Synergy of β -Lactams and Branched Polyethylenimine against MRSE & MRSA

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Abstract

Microbial biofilms are ubiquitous in nature, and they pose a serious threat to public health. *Staphylococcus epidermidis* and *S. aureus* is the most common clinical isolate from healthcare- and medical device-related biofilm infections. No antibiotic currently on the market can eradicate pathogenic biofilms, which contain complex defense mechanisms composed of slime-like extracellular polymeric substances (EPS). Understanding the need to develop alternative approaches, we examine 600-Da branched polyethylenimine (BPEI) against methicillin-resistant *Staphylococcus epidermidis* (MRSE) and *S. aureus* (MRSA) biofilms. Here, a microtiter biofilm model is used to test the synergistic effects between the two components of our combination treatment: BPEI and β -lactam antibiotics. Electron microscopy was used to confirm the growth of bacterial biofilms from the model. Minimum biofilm eradication concentration (MBEC) assays, crystal violet assays, and biofilm kill curves suggest that BPEI exhibits antibiofilm activity and can potentiate β -lactams to eradicate MRSE/MRSA biofilms.

Figure 1. Biofilm-disrupting ability of BPEI. BPEI disrupts established biofilm by collapsing the structure of EPS, releasing the cells into planktonic stage so that β -lactam antibiotics can actively target and eradicate them.

Introduction

Bacterial biofilms that are impenetrable to antibiotics pose an even greater threat when they are created by drug resistant bacteria, such as methicillin-resistant *S. epidermidis* & *S. aureus* (MRSE/MRSA). MRSA, MRSE, and their biofilms lead to chronic wound infections (i.e. wounds that have not proceeded through a reparative process in three months) that affect millions of Americans each year. With a dwindling arsenal of new antibiotics, existing drugs and regimens must be coupled with potentiators and re-evaluated as combination treatments for biofilms and antibiotic-resistant diseases. Our lab previously examined the efficacy of branched polyethylenimine (BPEI), a cationic polymer with a low molecular weight of 600-Da, against planktonic MRSE/MRSA bacteria. Cationic BPEI, with many primary and secondary amine groups, indirectly targets the resistant factor PBP2a by electrostatically binding to anionic wall teichoic acid required for PBP2a activity. In this manner, BPEI disabled resistance in MRSE/MRSA strains, restoring their susceptibility to traditional β -lactam antibiotics. In this study, we further investigate the application of our technology to MRSE/MRSA biofilms where the EPS contains numerous anionic biomacromolecules. The EPS creates a hydrophobic barrier that increases the tolerance of bacteria towards antimicrobials by reducing or preventing the antibiotics from reaching their target(s). When BPEI binds to EPS, two changes will occur. First, the anionic character of the biofilm will be neutralized leading to a disruption of the EPS matrix. Secondly, because BPEI is a hydrophilic polymer, it will disrupt hydrophobic barriers that hinder diffusion of aqueous antibiotics. Thus, BPEI functions as a potentiator by inhibiting and disrupting the EPS matrix allowing antibiotics to enter and kill the bacteria.

Experimental Methods

Experimental procedures were followed the methods of Lam *et al.*¹

Figure 2. A schematic experimental procedure of our microtiter biofilm model for synergistic effect screening against MRSE/MRSA biofilms.

Results and Discussion

Figure 3. SEM images of the tip of MBEC prongs. A control prong (A). MRSE 35984 biofilm colonies were formed after 24 hours of inoculation (B); the arrows highlight some of the biofilm microcolonies. Scale bars = 200 μ m.

Figure 4. SEM images of the MRSE 35984 biofilm. The intercellular matrices of EPS are captured as they wrap around every bacterium (A). At higher magnification (B). Scale bars = 2 μ m.

Figure 6. Established MRSE 35984 biofilms stained with crystal violet were treated with 600-Da BPEI for 20 hours. The dissolved biofilm solutions were transferred to a new plate, and the biofilm remainders are shown as top-down view (A). The mean OD550 of the dissolved biofilms was measured (B). Error bars denote standard deviation (n = 10). (t-Test, p-value < 0.01, significant difference between the negative control and each treatment is indicated with an asterisk).

Figure 8. Strong antibiofilm formation synergy between BPEI and piperacillin was observed, compared to individual piperacillin or BPEI treated samples. Error bars denote standard deviation (n = 3). PIP, piperacillin.

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Figure 5. Synergistic effects of BPEI and antibiotics against MRSE 35984 (A) and MRSE 29887 (B) on a 96-well checkerboard pattern. The synergy was seen both on the planktonic challenge plates (Aa and Ba) and the biofilm MBEC assays (Ab and Bb).

Figure 7. Biofilm kill curve of MRSE 35984. Only the combination treatment of BPEI+oxacillin (64 μ g/mL + 16 μ g/mL) – the diamond-curve – could eradicate MRSE 35984 biofilms. Error bars denote standard deviation (n = 2). CFU, colonies forming units.

Figure 9. MRSE 35984 biofilms (3-day old). The untreated control sample shows thick EPS enfolding every bacterial cell (A). BPEI-treated sample shows disrupted EPS and significant number of exposed cells without the EPS (B). At lower magnification, the untreated control (C) biofilms appear with full and tightly occupied biofilms, while the BPEI-treated sample (D) shows disjointed biofilms by many revealed surfaces. Scale bars (A and B) = 1 μ m. Scale bars (C and D) = 100 μ m.

Table 1: Synergistic effects between BPEI and ampicillin against MRSA biofilms

Strain	BPEI (μ g/mL)		Ampicillin (μ g/mL)			FICI	Synergy?
	MIC	MBEC	MIC	MBEC	MBEC + 64 μ g/mL BPEI		
MRSA 43300	64	>256	128	>256	2	0.13	yes
MRSA OU 6	>256	>256	256	>256	64	0.25	yes
MRSA OU 11	>256	>256	128	>256	32	0.19	yes

Figure 10. Established MRSA OUE biofilms treated with polymyxin B (PmB) and 600 Da BPEI for 20 hours, as well as the negative control (water only) and positive control (30% acetic acid). The dissolved biofilm solutions were transferred to a new plate, and the biofilm remainders are shown as top-down view (A). The mean OD550 of the dissolved biofilm solution was measured (B). Error bars = standard deviation (n = 10).

What's next?!

Broadening the antimicrobial spectrum of BPEI to potentiate **non- β -lactams** and against drug-resistant **Gram-negative** bacteria (*Pseudomonas aeruginosa* and *E. coli*)

References

- Lam, A. K.; Wouters, C. L.; Moen, E. L.; Pusavat, J.; Rice, C. V., Antibiofilm Synergy of beta-Lactams and Branched Polyethylenimine against Methicillin-Resistant *Staphylococcus epidermidis*. *Biomacromolecules* **2019**, *20* (10), 3778-3785.
- Lam, A. K.; Hill, M. A.; Moen, E. L.; Pusavat, J.; Wouters, C. L.; Rice, C. V., Cationic Branched Polyethylenimine (BPEI) Disables Antibiotic Resistance in Methicillin-Resistant *Staphylococcus epidermidis* (MRSE). *ChemMedChem* **2018**, *13* (20), 2240-2248.
- Lam, A. K *et al.* (2020) Low-molecular-weight Branched Polyethylenimine Potentiates Ampicillin against MRSA biofilms. *ACS MedChem Letters* DOI: 10.1021/acsmchemlett.9b00595