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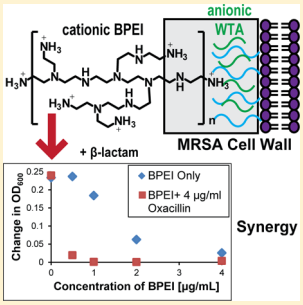
Targeting Wall Teichoic Acid *in Situ* with Branched Polyethylenimine Potentiates β-Lactam Efficacy against MRSA

Melissa A. Foxley, Summer N. Wright, Anh K. Lam, Anthony W. Friedline, Stoffel J. Strange, Min T. Xiao, Erika L. Moen, and Charles V. Rice*

Department of Chemistry and Biochemistry, Stephenson Life Sciences Research Center, University of Oklahoma, 101 Stephenson Parkway, Norman, Oklahoma 73019, United States

Supporting Information

ABSTRACT: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a medical concern. Here, we show that branched polyethylenimine (BPEI), a nontoxic, cationic polymer, restores MRSA's susceptibility to β-lactam antibiotics. Checkerboard assays with MRSA demonstrated synergy between BPEI and β-lactam antibiotics. A time-killing curve showed BPEI to be bactericidal in combination with oxacillin. BPEI did not potentiate efficacy with vancomycin, chloramphenicol, or linezolid. When exposed to BPEI, MRSA increased in size and had difficulty forming septa. BPEI electrostatically binds to wall teichoic acid (WTA), a cell wall anionic polymer of Gram-positive bacteria that is important for localization of certain cell wall proteins. Lack of potentiation in a WTA knockout mutant supports the WTA-based mechanism. These data suggest that BPEI may prevent proper localization of cell wall machinery by binding to WTA; leading to cell death when administered in combination with β-lactam antibiotics. Negligible *in vitro* toxicity suggests the combination could be a viable treatment option.



KEYWORDS: *Branched polyethylenimine, MRSA, wall teichoic acid, bacteria, antibiotic resistance, β-lactams*

Clinicians prescribed 118 million courses of β-lactam antibiotics in 2011 to treat the majority of patients presenting bacterial infection symptoms.[1] Patient outcomes are generally positive unless β-lactam resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), are present. In cases where symptoms are attributed to MRSA, β-lactams are avoided and effective antibiotics are given without delay.[2] However, most MRSA infections are not diagnosed immediately.[3] After initial infection symptoms arise, patients suffer while ineffective antibiotics, usually β-lactams, are given.[3] MRSA colonies survive and invade host tissue to release toxins that cause tissue injury. Therefore, the need exists to decrease morbidity and mortality while improving the quality of life and medical outcomes of patients infected by MRSA. These needs can be met by strengthening the arsenal of clinical first-line antibiotics and using these new weapons to fight MRSA. A new antibiotic treatment option with efficacy against MRSA and MSSA could be based on branched polyethylenimine (BPEI) and β-lactams.

β-Lactam antibiotics, such as oxacillin and ampicillin, prevent bacterial cell wall synthesis by irreversibly binding to the active site of penicillin-binding proteins (PBPs) that are responsible for cross-linking peptidoglycan. MRSA has the *mecA* gene that codes for PBP2a, an extra PBP with a low binding affinity for β-lactam antibiotics, thereby reducing their effectiveness in treatment.[4] PBP4 has also been shown to aid community-acquired MRSA in β-lactam resistance.[5] Wall teichoic acid (WTA) is important for proper function and localization of PBP2a and PBP4.[6−8] As an anionic polymer found in the cell wall of Gram-positive bacteria, WTA is also important for metal binding, cell adhesion, virulence, biofilm formation, and localization of autolysins.[9−14] As inhibition of WTA synthesis makes MRSA susceptible to β-lactam antibiotics,[7,8] similar potentiation effects could arise by disrupting WTA function with BPEI.

An initial study published by our lab found that BPEI and ampicillin had synergy against MRSA, which we proposed was due to electrostatic binding of BPEI to WTA.[15] Expanding on our initial study, we investigated the anti-MRSA properties of BPEI and discovered that small amounts (<1 μg/mL, <1.6 μM) of BPEI potentiate β-lactam antibiotics against MRSA. We found that BPEI has synergy with oxacillin, ampicillin, and amoxicillin against MRSA. BPEIs of different molecular weights were tested, but due to toxicity concerns of the higher Dalton BPEIs, the lowest MW (600 Da BPEI) was considered to be the lead potentiator. Additionally, BPEI increased cell size and reduced autolytic rates. Microscopy images show perturbation of the cell wall while time−kill curves demonstrate that the combination of oxacillin and BPEI is bactericidal. Also, the minimum bactericidal concentration (MBC) for the combination is less than four-fold higher than the minimum inhibitory concentration (MIC), which indicates a bactericidal mechanism. We believe that BPEI works by causing steric hindrance of WTA to prevent proper localization of PBP2a and PBP4, which effectively disables this β-lactam resistance factor.

BPEI's value in drug discovery is supported by mammalian cell cytotoxicity assays that demonstrate that 600 Da BPEI does not

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Table 1. Synergy of 600 Da BPEI and β-Lactams against MRSA 700787 and MSSA 25923[a]

		MIC_A (μg/mL)/μM		MIC_B (μg/mL)/μM			
strain	antibiotic	alone	comb.	alone	comb.	FICI	outcome
MRSA 700787	oxacillin	64/149	4/9	4/6.6	0.5/0.8	0.188	synergy
			0.5/1.1		1/1.6	0.258	
	ampicillin	128/345	8/22	4/6.6	0.5/0.8	0.188	synergy
			2/5		1/1.6	0.266	
	amoxicillin	128/305	8/22	4/6.6	1/1.6	0.313	synergy
	methicillin	64/160	4/10	4/6.6	2/3.3	0.563	additivity
	meropenem	2/4.6	0.5/1.1	4/6.6	0.5/0.8	0.375	synergy
	chloramphenicol	8/25	2/6	4/6.6	1/1.6	0.500	additivity
	linezolid	2/5.9	0.5/1.5	4/6.6	1/1.6	0.500	additivity
	vancomycin	2/1.3	2/1.3	4/6.6	4/6.7	1.0	no synergy
MSSA 25923	oxacillin	0.064/0.14	0.032/0.072	32/53	8/13	0.75	additivity
	ampicillin	0.064/0.17	0.032/0.086	32/53	8/13	0.75	additivity
	amoxicillin	0.064/0.16	0.016/0.038	32/53	16/27	0.75	additivity

[a]MIC_A is minimum inhibitory concentration of the antibiotic; MIC_B is minimum inhibitory concentration of BPEI; FICI is fractional inhibitory concentration index.

impact viability until concentrations are much higher than those required for *in vitro* efficacy. BPEI may have oral bioavailability because BPEI is a hydrophilic weak base with high water solubility; whose absorption by the gut epithelium was demonstrated by Cheng et al. *in vitro* using Caco-2 epithelial cells and an *in vivo* rat model.[16] Future animal studies will test this hypothesis for BPEI + antibiotic combinations. Enormous benefits to patient health are possible by providing clinicians with improved first-line antibiotics to lower health care costs, decrease morbidity, and reduce the need for MRSA screening. We recognize that a large amount of work remains in order to demonstrate *in vivo* efficacy, safety, bioavailability, and optimal drug formulation. Yet, the biophysical properties of BPEI and the data generated in our laboratory provide guarded optimism that we can successfully address these concerns and overcome obstacles within the drug discovery pipeline.

MRSA is a concern for human health due to its ability to infect humans and its resistance to commonly used β-lactam antibiotics. The CDC considers MRSA to be a serious threat to public health. It is estimated that 1 in 7 severe cases of MRSA resulted in death in 2011.[17] Additionally, many patients with MRSA undergo surgery to remove infected tissue. Drugs of last resort, such as vancomycin, require hospitalization. Meanwhile, strains of MRSA resistant to vancomycin, daptomycin, and linezolid have been found.[18−21] Orally available β-lactams, the most common class of first-line antibiotics,[1] could be used in a new anti-MRSA drug combination by overcoming a leading cause of β-lactam antibiotic resistance in MRSA: PBP2a and PBP4.[5,22] Disabling PBP2a and PBP4 with oral β-lactams has not reached clinical use, but success may be possible with a discovery made in our laboratory.[15] β-Lactam antibiotics that kill MSSA are potentiated to stop MRSA growth if coadministered with 600 Da BPEI, which is noncytotoxic.

BPEI creates synergy with β-lactam antibiotics to stop MRSA growth. Our previously published work showed that BPEI potentiated ampicillin susceptibility in MRSA.[15] Additional checkerboard assays demonstrate anti-MRSA properties of 600 Da BPEI mixed with β-lactam antibiotics (Table 1) against a MRSA strain containing PBP2a (ATCC 700787) that is also moderately resistant to vancomycin (vancomycin-intermediate *S. aureus*, VISA). BPEI has synergy (FIC < 0.5) with oxacillin, ampicillin, and amoxicillin (Figure 1A−C). Adding 600 Da BPEI (0.5 μg/mL, 0.8 μM) reduced the MIC of oxacillin from 64 μg/mL (149 μM) to 4 μg/mL (9 μM) against MRSA 700787. Increasing the concentration of 600 Da BPEI to 1 μg/mL (1.6 μM) resulted in a 128-fold decrease of the MIC of oxacillin to 0.5 μg/mL (1.1 μM). Likewise, increasing the 600 Da BPEI concentration increases potentiation and decreases MIC values of amoxicillin and ampicillin (Table 1, Figure 1B,C). BPEI did

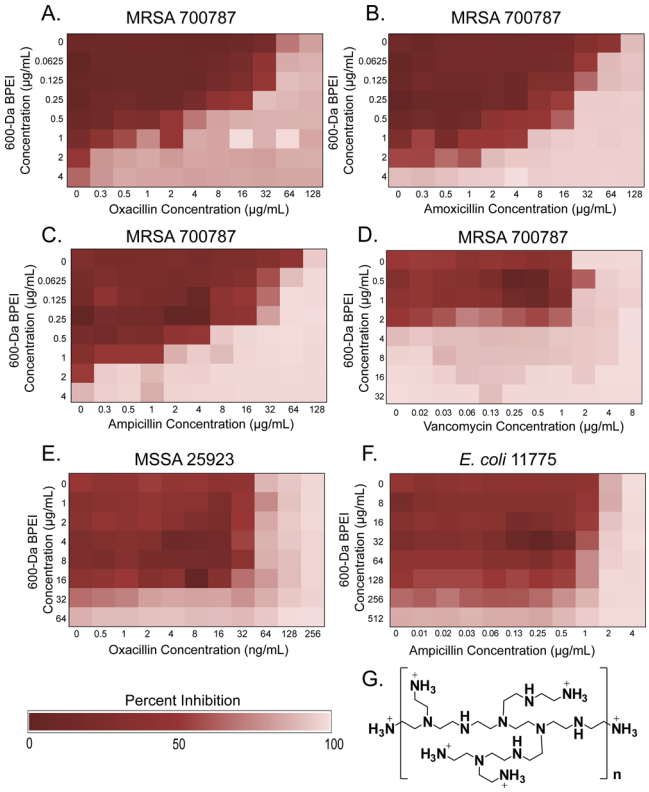

Figure 1. BPEI potentiates β-lactam activity against MRSA 700787, but not MSSA 25923 or *E. coli* 11775. Checkerboard assays show that BPEI potentiates oxacillin (A), amoxicillin (B), and ampicillin (C) activity against MRSA 700787. BPEI does not potentiate vancomycin (D) activity. No synergy is seen between β-lactams and BPEI against MSSA 25923 (E) or *E. coli* 11775 (F). Each assay was performed as three separate trials, and the presented data is shown as the average change in OD_{600}. The structure of BPEI (G) is shown (n = 1, MW = 600 Da; n = 2, MW = 1200 Da; n = 3, MW = 1800 Da).

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not have synergy with non-β-lactam antibiotics, such as vancomycin, linezolid, and chloramphenicol (Table 1, Figure 1D). Additionally, BPEI only had modest additivity with oxacillin (64 ng/mL, 140 nM, Figure 1E), ampicillin (64 ng/mL, 170 nM, Figure S1A), and amoxicillin (64 ng/mL, 160 nM, Figure S1B) against MSSA (ATCC 25923) (Table 1). The different BPEI potentiation effects against MRSA versus MSSA support a mechanism involving PBP2a, which is present in MRSA 700787 but not in MSSA. We also report that the combination of BPEI and β-lactam antibiotics does not have synergy against Gram-negative *E. coli* 11775 (Figure 1F) or Gram-positive *B. subtilis* 6051 (Figure S1F). These data lead us to believe that the mechanism by which BPEI potentiates β-lactams is specific for MRSA.

BPEI is commercially available in a wide range of sizes (600 to 1 000 000 Da). We were motivated to determine if increasing molecular weight of BPEI increases efficacy against MRSA. The MICs of 1200 and 1800 Da BPEI alone (0.5 μg/mL, 0.42 μM, and 0.28 μM, respectively) were lower compared to that of 600 Da BPEI alone (4 μg/mL, 6.6 μM). The 1200 and 1800 Da BPEI had synergy with oxacillin (Figure S1C,D). Only 0.125 μg/mL (0.10 μM) 1200 Da BPEI was required to reduce the MIC of oxacillin from 64 μg/mL (149 μM) to 4 μg/mL (9 μM) against MRSA 700787. The 10 000 Da BPEI had an MIC of 4 μg/mL (0.4 μM) but did not demonstrate synergy with oxacillin (Figure S1E). Compared to the 600 Da BPEI+ β-lactam combination, the 1200 and 1800 Da + β-lactam combinations have superior *in vitro* efficacy. However, as shown below, 600 Da BPEI shows negligible *in vitro* cytotoxicity against mammalian cells, whereas toxicity increases for 1200, 1800, and 10 000 Da BPEI.

The 600 Da BPEI has low cytotoxicity. A possible critique of utilizing BPEI in drug discovery is that cationic BPEIs are cytotoxic and thus unlikely to be useful as clinical antibacterial treatments. Reports show that cationic compounds, such as aminoglycosides and polymyxins, lead to nephrotoxicity.[23,24] However, presumptions that all BPEIs are toxic overlook the stipulation that toxicity depends on molecular weight and concentration. High molecular weight BPEIs (over 25 000 Da) are cytotoxic, whereas the lower molecular weight BPEIs are nontoxic unless their concentrations are orders of magnitude higher than the amount required for potentiation (Table 1).[25,26] Low cytotoxicity was confirmed in our lab with *in vitro* cytotoxicity data using mouse fibroblasts,[15] human HeLa, humancolon, and human kidney cell lines (Table 2). The IC_{50} values for 600 Da BPEI (100−1000 μg/mL, 166−1670 μM) are orders of magnitude higher than the amount required for potentiation (~1 μg/mL, 1.6 μM). An *in vitro* hemolysis assay, published by Gibney et al., showed that 600 Da BPEI had no hemolytic activity and that 10 000 Da BPEI had minimal hemolysis (>5%) up to 2000 μg/mL.[25] An *in vitro* nephrotoxicity assay was performed using primary human renal proximal tubule epithelial cells (hRPTECs). The assay detects the release of the metabolic enzyme, lactate dehydrogenase (LDH). A lack of LDH release suggests that the membrane is not damaged and that the test agent does not cause *in vitro* nephrotoxicity. Exposure to 600 Da BPEI caused minimal release of LDH (~1% at 8 μg/mL [13 μM], 16 μg/mL [27 μM], and 31 μg/mL [52 μM]; 3.5% at 62 μg/mL [103 μM]; and 8% at 125 μg/mL [208 μM], Figure 2). As

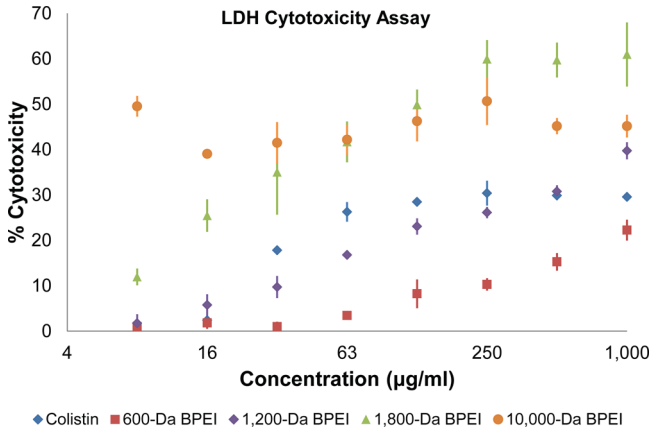

Figure 2. The 600 Da BPEI had minimal LDH release in a primary kidney proximal tubule cell line (PCS-400-010). The 1800 and 10 000 Da BPEIs had higher LDH release than the nephrotoxic drug colistin. Error bars denote standard deviation (n = 3).

per the literature, these values are indicative of low toxicity.[27] These values are much lower than the LDH release values for cationic and nephrotoxic colistin (1% at 8 μg/mL [7 μM]; 2.3% at 16 μg/mL [14 μM]; 18% at 31 μg/mL [27 μM]; 26% at 62 μg/mL [54 μM]; and 28% at 125 μg/mL [108 μM], Figure 2). The 1200, 1800, and 10 000 Da BPEIs were more toxic than the 600 Da BPEI as seen with the CellTiter Blue cell viability (Table 2) and LDH assays (Figure 2). These data suggest 600 Da BPEI is preferred as a lead potentiator in drug discovery due to its low toxicity and low nephrotoxicity.

The low toxicity of our lead potentiator is the result of its hydrophilic nature. The 600 Da BPEI is very hydrophilic and completely miscible with water. Also, 600 Da BPEI molecules are very small and do not contain regions of hydrophobic character, as seen with cationic peptides, aminoglycosides, and polymyxins. Thus, 600 Da BPEI lacks the energetic force that drives hydrophobic compounds into lipid membranes. Conversely, the higher MW BPEIs possess hydrophobic interiors that increase their lipophilicity and lead to membrane penetration and damage and, ultimately, higher toxicity.

The 600 Da BPEI:oxacillin combination is bactericidal. Monitoring the growth of MRSA 700787 reveals that bacteria exposed to subinhibitory concentrations of BPEI and oxacillin fail to reach the exponential phase when the two compounds are combined (Figure 3A). Aliquots were transferred to agar plates, and CFUs were counted from the 0, 4, 8, and 24 h time points to determine if the lack of growth observed for the BPEI and oxacillin combination was bacteriostatic or bactericidal. After 8 h, the control sample had the highest cell density (1×10^9 cells/μL). The samples that contained BPEI or oxacillin had lower viable cell numbers in comparison to the control sample, but these values were still higher than the initial cell density (>1×10^5 cells/μL). The viable cell count decreased for growth in both

Table 2. *In Vitro* Mammalian Cytotoxic Activity of BPEI[a]

	mean IC_{50} ± SD (μM)			
human cell line	600 Da	1200 Da	1800 Da	10 kDa
cervical cancer (HeLa)	1820 ± 250	692 ± 13	519 ± 34	0.66 ± 0.089
colon cancer (HCT116)	487 ± 75	457 ± 48	100 ± 42	1.11 ± 0.22
kidney cancer (HEK293)	1150 ± 130	155 ± 23	33 ± 13	0.19 ± 0.05

[a]Values are reported as the average of three trials ± standard deviation. Cells were treated for 48 h and assayed with the CellTiter Blue method.

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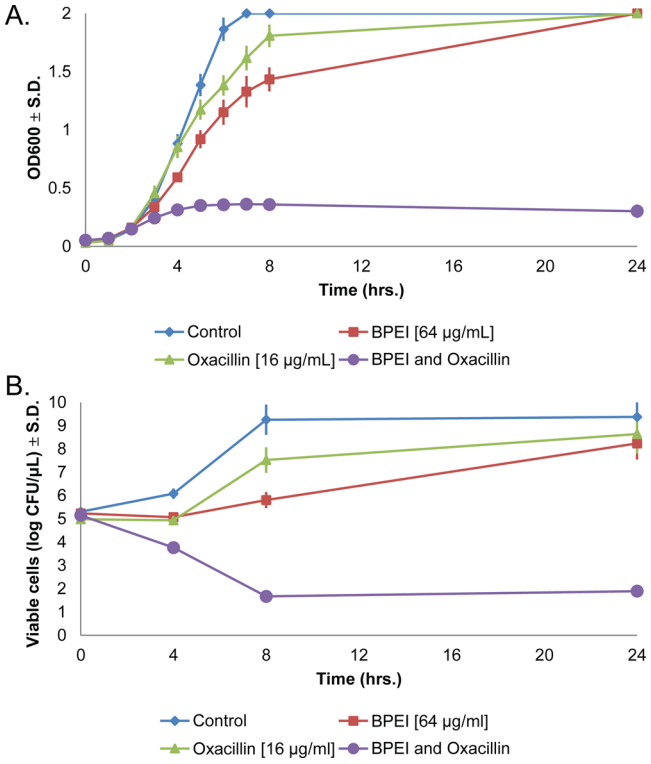

Figure 3. The 600 Da BPEI and oxacillin comprise a bactericidal combination in MRSA 700787. Cells treated individually with BPEI or oxacillin were still able to grow, but growth was inhibited when both BPEI and oxacillin were used (A). A time–kill curve shows that the combination has a bactericidal mechanism of action as seen with the drop in viable cell counts after 4 h (B). Error bars denote standard deviation (n = 3).

BPEI and oxacillin (47 CFUs/μL at 8 h growth) and did not increase after 24 h (Figure 3B). This data demonstrates that the mechanism by which BPEI and oxacillin prevents growth of MRSA is bactericidal. To support this conclusion, MBCs were determined from a checkerboard assay of MRSA containing BPEI and oxacillin. The MBC of BPEI was the same as the MIC (4 μg/mL, 6.7 μM), while the MBC for oxacillin was at least twice as large as the MIC (MIC = 64 μg/mL, 149 μM; MBC > 128 μg/mL, 290 μM). The MBCs for the combination (1 μg/mL BPEI, 2 μg/mL oxacillin; Figure S2) were two-fold greater than the combination MICs (1 μg/mL BPEI, 1 μg/mL oxacillin). If the MBC is less than four-fold of the MIC, the drug is considered bactericidal and does not require the assistance of host defenses.[28] Since the MBC for BPEI and its oxacillin combination is not greater than four times the MIC, both BPEI alone and the BPEI–oxacillin combination are bactericidal.

WTA is important for BPEI potentiation of β-lactam antibiotics. Studies have shown that WTA is important for β-lactam resistance in MRSA and aids the localization of PBP4 to the division septum.[7,8,29,30] WTA is dispensable for cell growth; however, WTA deficient mutants of MRSA have an increased susceptibility to β-lactam antibiotics.[7] Also, by inhibiting WTA synthesis by chemical inhibition of TarO, the protein responsible for the first step of WTA synthesis results in a decreased resistance. Recent work has been done to develop new WTA inhibitors to fight MRSA with reduced protein binding effects.[30−32] Removal of WTA through genetic or chemical means reduced CFUs in mice treated with imipenem.[29] Disabling mature WTA in the cell wall should have a similar effect.

BPEI, with its polycationic properties, has the potential for very strong electrostatic interactions with the polyanionic WTA molecules. Localization of BPEI to the Gram-positive cell wall results from interactions between the primary amines of BPEI and the phosphate groups of WTA. Previously, using laser scanning confocal microscopy, we showed that BPEI tagged with Alexa Fluor 488 localizes on the perimeter of Gram-positive MRSA cells but not on Gram-negative *E. coli* cells.[15] Using NMR, we further found that phosphate–amine binding from the WTA–BPEI interactions likely occurs through electrostatic attraction between the numerous cationic primary amines of BPEI and anionic phosphate groups of WTA.[15] BPEI and ampicillin have synergy against MRSA MW2 (Figure S3A). However, BPEI did not exhibit synergy with ampicillin against MRSA MW2 Δ*tarO* (strain referenced in ref 7), which lacks WTA (Figure S3B). This, combined with the data in Figure 1, suggests that BPEI potentiation arises through binding to WTA, preventing proper localization of PBP2*a*/4, and thereby allowing cell death via β-lactam inhibition of other PBPs.

BPEI prevents proper cell division in MRSA. WTA deficient mutants have altered morphologies. SEM images at midexponential phase showed that a WTA-deficient mutant was significantly larger and had a rougher surface than the wild-type cells.[33] We were motivated to determine if BPEI caused any morphological changes in MRSA. MRSA cells were grown in sublethal concentrations of BPEI, isolated at late-lag phase, and imaged. SEM images show that BPEI does not dramatically alter the shape of MRSA 700787. However, the size of the cells was significantly increased (p-value < 0.001) from 0.86 ± 0.11 to 0.98 ± 0.14 μm when grown in BPEI, and the numerous MRSA aggregates suggest the inability to properly form septa and complete the cell division process (Figure 4). The cell

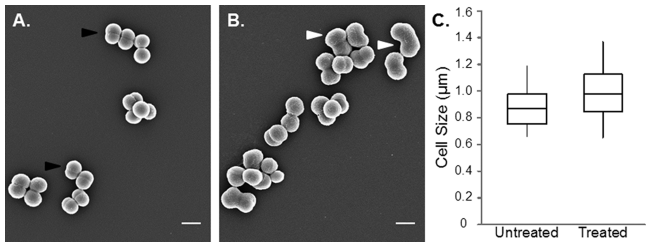

Figure 4. BPEI caused a significant increase in MRSA 700787 cell size. The average cell size (largest diameter) for untreated cells is 0.86 ± 0.11 μm (A) but increases to 0.98 ± 0.14 μm when grown in 64 μg/mL (107 μM) BPEI (B). Normal cleavage furrows are shown with black arrows. Enlarged cells that do not have cleavage furrows present are shown with white arrows. Cells were fixed and imaged at late-lag phase. Scale bar equals 1 μm. A size analysis graph (C) shows the average cell size (center line), the standard deviation (outside of box), and minimum and maximum values (error bars) of 100 measured cells (p-value < 0.001).

morphologies do not appear to be significantly altered; however, the cleavage furrow does not form properly for the cells treated with BPEI. The phenotypic similarity between cells grown in BPEI and knockout mutant cells suggests that the altered morphologies may arise from an interaction between BPEI and WTA. If so, autolysin activity, which is controlled by WTA, should also be affected.

Autolysins are used by bacterial cells to lyse peptidoglycan strands during cell division, allowing separation of daughter cells. The importance of WTA and lipoteichoic acid (LTA) in the

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regulation of autolysin activity is well-known. Schlag et al. found that autolysis increased in WTA knockout mutants and proposed that mature WTA prevents binding of Atl, an autolytic protein in *S. aureus*, to the mature cell wall.[33] WTA knockout mutants were unable to correctly localize Atl to the division septum.[33] Campbell et al. suggests that, when WTA is absent, inefficient septal formation occurs due to the degradation of the autolysins track that allows for cell separation.[7] Our SEM images show that MRSA aggregates arise after BPEI exposure, which could have resulted if autolysis was hindered (Figure 4). Triton X-100 induced autolytic rate measurements were performed to determine if BPEI affects autolysis in MRSA 700787. The autolytic rate of MRSA cells grown in 16 μg/mL (27 μM) of BPEI was slowed in comparison to control samples (Figure S4). Autolysis was completely stopped for MRSA cells grown in 64 μg/mL (107 μM) of BPEI. Due to a larger initial cell density ($\sim 1 \times 10^8$ CFUs/mL), the concentration of BPEI used was higher than the MIC found in the checkerboard assays ($\sim 5 \times 10^5$ CFUs/mL). Triton X-100 induced autolysis is believed to occur via the release of LTA from the cell wall.[34] The origin of BPEI-induced slowing of autolysis is unknown, but a possible explanation is the prevention of LTA release by the electrostatic binding of BPEI to LTA, which causes steric restraint and prevents Atl localization. Further work is necessary to test this hypothesis.

Diagnosed or suspected MRSA infections require treatment with vancomycin, linezolid, or daptomycin;[2] and to date, no MRSA strain is resistant to more than one of them.[20,35] Other drugs such as ceftaroline, teflaro, and telavancin have been approved for patient use in severe cases, but all must be given intravenously.[2] Yet, people suffer from MRSA infections because MRSA is either misdiagnosed or not suspected, and ineffective first-line antibiotics, usually β-lactams,[1] are given. After MRSA diagnosis, clinicians turn to drugs of last resort, but treatment delays can result in mortality or increased morbidity due to release of MRSA toxins into tissue.[35,36] In 2011, MRSA infected 80 500 people; nearly 1 in 7 cases resulted in death (11 300; 14%).[17] Our expected research contribution is to address these drawbacks by blocking the PBP2a and PBP4 resistance pathways with BPEI, thereby potentiating β-lactam antibiotics to kill MRSA. The typical route is inhibiting WTA synthesis to prevent PBP2*a*/4 function. Here, we have described a different approach that, in our opinion, departs from the status quo by deactivating mature WTA within the cell wall through electrostatic interactions with BPEI.[15] Nontoxic 600 Da BPEI was able to potentiate β-lactam efficacy against MRSA. The mechanism of action is electrostatically binding to WTA and thereby preventing proper localization of PBP2a/PBP4 (Figures S5 and S6). Future studies are planned to probe the structure–activity relationship, test *in vivo* efficacy, and further elucidate the mechanism of action. The impact of our contribution is meeting the need for new first-line antibiotics that can be given promptly at the first sign of any *S. aureus* infection to kill MSSA and/or MRSA without the need to identify MRSA as the infectious agent.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00285.

Additional figures and experimental section (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: rice@ou.edu.

ORCID

Charles V. Rice: 0000-0003-1424-405X

Author Contributions

The manuscript was written through contributions of all authors. All authors have approved the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; BPEI, branched polyethylenimine; NMR, nuclear magnetic resonance; SEM, scanning electron microscopy; WTA, wall teichoic acid; LTA, lipoteichoic acid; PBP, penicillin-binding protein; FIC, fractional inhibitory concentration; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; OD_{600}, optical density at 600 nm; Da, Dalton; MW, molecular weight; LDH, lactate dehydrogenase

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