SUPPORTING INFORMATION

LOW-MOLECULAR-WEIGHT BRANCHED POLYETHYLENIMINE POTENTIATES AMPICILLIN AGAINST MRSA BIOFILMS

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EXPERIMENTAL PROCEDURE

Materials

In this experiment, the *Staphylococcus aureus* (MRSA 43300) was purchased from the American Type Culture Collection. Two MRSA clinical isolates (MRSA OU6 & OU11) from patient swabs were kindly provided by Dr. McCloskey from the University of Health Sciences Center with an institutional review board (IRB) approval. Chemicals (DMSO, growth media, and electron microscopy fixatives) were purchased from Sigma-Aldrich. Antibiotics (ampicillin and polymyxin B) were purchased from Gold Biotechnology. 600 Da BPEI was purchased from Polysciences. MBEC™ Biofilm Inoculators were purchased from Innovotech. Isopore polycarbonate membrane filters (0.1 µm pore size, hydrophilic, 13 mm diameter) were purchased from MilliporeSigma.

MBEC Assay

This method is adapted from our previous study.[1] In brief, bacterial culture was inoculated in an MBEC pronged-inoculator and incubated for 24 hr to allow *biofilm formation*. Then, the preformed biofilm prong lid was washed and treated in a separate *challenge plate* which was prepared as a checkerboard assay:[2] serial dilutions of BPEI and antibiotic solutions were added to a 96-well base plate with a total volume of 200 µL cation-adjusted Muller Hinton broth (MHB) per well. The change in optical density at 600 nm (ΔOD_{600}) was measured. Minimum inhibitory concentration (MIC) of each drug is determined as the lowest concentration that inhibited cell growth ($\Delta OD_{600} < 0.05$). Fractional inhibitory concentration index (FICI) was calculated as: $FICI = \frac{MIC_{AB}}{MIC_A} + \frac{MIC_{BA}}{MIC_B}$. Synergistic effects are determined using EUCAST guidelines: synergy (FICI ≤ 0.5), additivity (0.5 < FICI < 1), and indifference (FICI > 1).[3] The treated pronged-inoculator was then washed and transferred to a *recovery plate* with 200 µL MHB/well to sonicate and recover any remaining biofilm bacteria. The recovery plate was then incubated overnight before measuring ΔOD_{600} to determine MBECs and FICIs of the drugs tested on the biofilms.

Biofilm Disrupting Assay

This method is also described in details by Lam et al.[1] This experiment was parallelly conducted with polymyxin B (PmB, a cationic polypeptide antibiotic) and BPEI. In short, an overnight MRSA OU 6 culture was inoculated in a tissue-culture treated 96-well plate (100 µL of tryptic soy broth or TSB/well) with an inoculation size of 1 µL/well (~5 x 10^5 CFU/mL). The plate was incubated at 35 ºC for 24 hr to allow the bacteria to form biofilm. It was then washed with water to remove planktonic bacteria and stained with 100 µL of crystal violet solution (0.1%) per well for 15 min. The stained plate was washed excessively with water 5 times to remove any unbound stain and air-dried overnight. Vary concentrations of PmB (64 and 128 µg/mL) and 600 Da BPEI (64 and 128 µg/mL) were added to the stained-biofilm plate with a total volume of 100 µL/well. A negative control (water only) and positive control (30% acetic acid) were also conducted at the same time of treatment. After 20 hr, without touching the biofilm layer in the bottom of the plate, solubilized solution in each treated well was carefully transferred to a new 96-well plate for an OD_{550} measurement, which represents the corresponding amount of biofilm disrupted by each treatment.

Scanning Electron Microscopy (SEM)

MRSA OU6 were inoculated from 0.5% of an overnight culture on glass coverslips and grown at 35 °C. After 24 hr. the biofilm-formed on glass coverslips were carefully removed and washed in water for 10 s. Then each sample was submerged in different treated solution (untreated control, 128 µg/mL BPEI-treated, and bleach-positive control) for another 24 hr. Next, they were removed, washed in water for 10 s, and submerged in primary fixative (5% glutaraldehyde in 0.1 M cacodylate buffer) and incubated at 4 ± 2 °C for 2 days. The glass coverslips were removed from the fixing solution and air-dried for 72 hr. They were mounted on aluminum stubs with carbon tape and sputter-coated with AuPd. A Zeiss NEON SEM was used to image the samples at 5 kV accelerating voltage.

SEM of Biofilms on Polycarbonate Membrane Filters

Pre-sterilized polycarbonate (PC) membranes were gently adhered to a tryptic soy agar plate using sterilized forceps. A volume of 2 µL of the stock MRSA OU6 solution (~5 x 10^5 CFU/mL) was pipetted on top of each PC membrane and incubated at 35 °C for 7-8 hr, when the MRSA biofilm colony on the PC membranes became visible to the naked eye. The PC membranes with preformed biofilm was then carefully removed off the agar, transferred into a treatment solution of 256 µg/mL BPEI, and incubated for another 20 hr. Untreated and treated PC samples were removed and washed in water for 10 s. They were submerged in primary fixative (5% glutaraldehyde in 0.1 M cacodylate buffer) and incubated at 4 ± 2 °C for 2 days. The PC samples were air-dried slowly for 3 more days. They were mounted on aluminum stubs with double-side carbon tape, sputter-coated with AuPd, and imaged at 5 kV accelerating voltage by a Zeiss Neon SEM.

MRSA Clinical Isolate Data Collected at OUHSC Clinical Microbiology Laboratory								
			Clindamycn		Daptomycin		Erythromycin	
Isolate	Species	Methicillin Resistant	MIC	Interp	MIC	Interp	MIC	Interp
6	S. aureus	Y	R	>4	S	≤0.5	R	>4
11	S. aureus	Y	R	>4	S	≤0.5	R	>4
Isolate	Species	Methicillin Resistant	Gentamicin		Linezolid		Oxacillin	
6	S. aureus	Y	MIC	Interp	MIC	Interp	MIC	Interp
11	S. aureus	Y	S	≤4	S	2	R	>2
			S	≤4	S	2	R	>2
Isolate	Species	Methicillin Resistant	Tetracycline		Trimeth/Sulfa		Vancomycin	
6	S. aureus	Y	MIC	Interp	MIC	Interp	MIC	Interp
11	S. aureus	Y	S	≤4	S	≤0.5/9.5	S	2
			S	≤4	S	≤0.5/9.5	S	1
Unless otherwise indicated, identificaiton and susceptibility performed by								
	the Beckman Coulter MicroScan Walkaway 96plus using the PC33 gram positive panel							
*Presumed resistant/D test (inducible clindamycin resistance) positive								
^Species identification and oxacillin/methicillin Susceptibility/Resistance determined								
	by Verigene Gram Positive Blood Culture assay (probes for Genus/species and mecA)							

Citations

1. 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.

2. 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.

3. European Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical, M.; Infectious, D., EUCAST Definitive Document E.Def 1.2, May 2000: Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. *Clin Microbiol Infect* **2000,** *6* (9), 503-8.