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Peptidoglycan-Directed Chemical Ligation for Selective Inhibition on Gram-Positive Bacteria

Feng Jiang, Chengteng Cai, Lei Gao, Xinhui Su,* and Shoufa Han*

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ABSTRACT: Microbicides with distinct antibacterial mechanisms show potential to combat multi-drug resistance bacteria. We herein report peptidoglycan-directed chemical ligation (PGCL) between alkyne-bearing vancomycin and an azide-tagged cationic polymer. The former binds peptidoglycan and inhibits peptidoglycan crosslinking, while the latter interferes the integrity of the bacterial membrane. PGCL results in enhanced bactericidal activity against Gram-positive *Staphylococcus aureus* (*S. aureus*) over Gram-negative *Escherichia coli* (*E. coli*). These data indicate the potential of PGCL to selectively and synergistically inhibit Gram-positive pathogens via dual modality antibacterial mechanisms of peptidoglycan-inhibiting antibiotics and bacterial membrane-disrupting polycations.
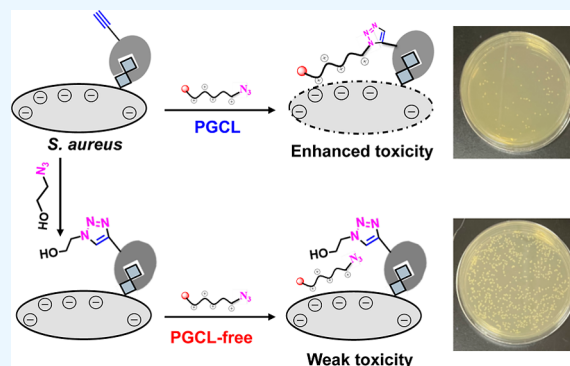
INTRODUCTION

Pathogenic bacteria such as *Staphylococcus aureus* cause diverse diseases including wound infection[1,2] and otitis media.[3−5] Antibiotics exhibit defined mechanisms to inhibit bacterial survival. For instance, vancomycin binds the D-alanyl-D-alanine (D-ala-D-ala) terminal of the pentapeptide and thus blocks peptidoglycan crosslinking.[6−10] Vancomycin was once regarded as the last line against drug-resistant Gram-positive bacteria.[11−13] However, high frequency use of antibiotics yields drug resistance. In this context, various vancomycin derivatives have been developed against vancomycin-resistance bacteria[6,14−22] Given the rise of drug-resistance *S. aureus* and other bacteria, there is an urgent need for new antibacterial approaches alternative to classical antibiotics.[23,24]

Cationic materials exhibit bactericidal effects on both Gram-positive and Gram-negative bacteria.[25−27] These polycations electrostatically bind to the negatively charged bacterial membrane, leading to decomposition of the cell membrane and bacterial death.[28−30] Given the broad spectrum microbial nature, polycations that could be exploited to selectively inhibit pathogenic bacteria are potential tools against drug-resistant bacteria. In addition, approaches that allows selective ablation of Gram-positive bacteria while sparing Gram-negative bacteria are of significance in diverse clinical settings including *S. aureus* and *Enterococcus faecalis*.[31−35]

On the basis of these considerations, we sought to combine vancomycin with polycationic materials to selectively inhibit Gram-positive bacteria via distinct antibacterial mechanisms. This is operated with the use of dibenzocyclooctyne (DBCO)-tagged vancomycin (DBCOVan) that binds to peptidoglycan and an azide-tagged polymeric quandary amine (AzPolyQa).

Binding of DBCOVan and AzPolyQa on peptidoglycan enables stain-promoted azide−alkyne cycloaddition (SPAAC) between AzPolyQa and DBCOVan (Figures 1 and 2). Compared to
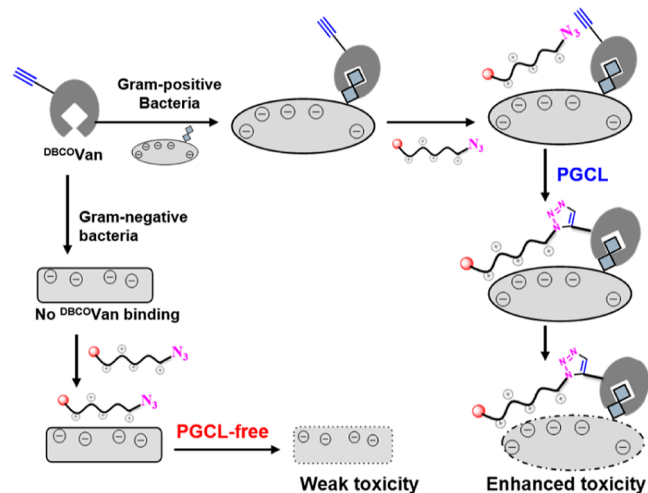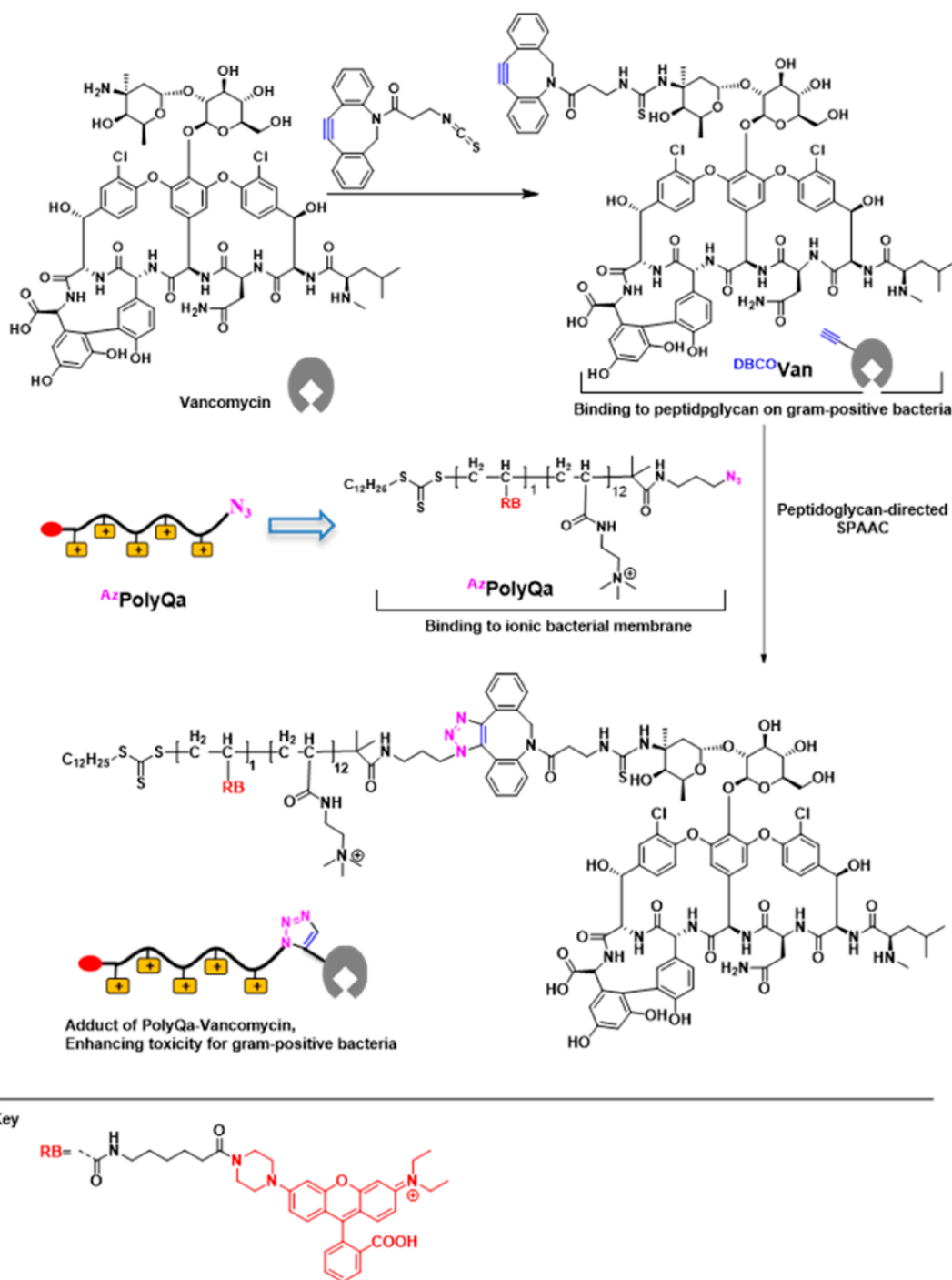

Figure 1. Schematic for PGCL synergistic antibacterial activity on Gram-positive bacteria over Gram-negative bacteria.

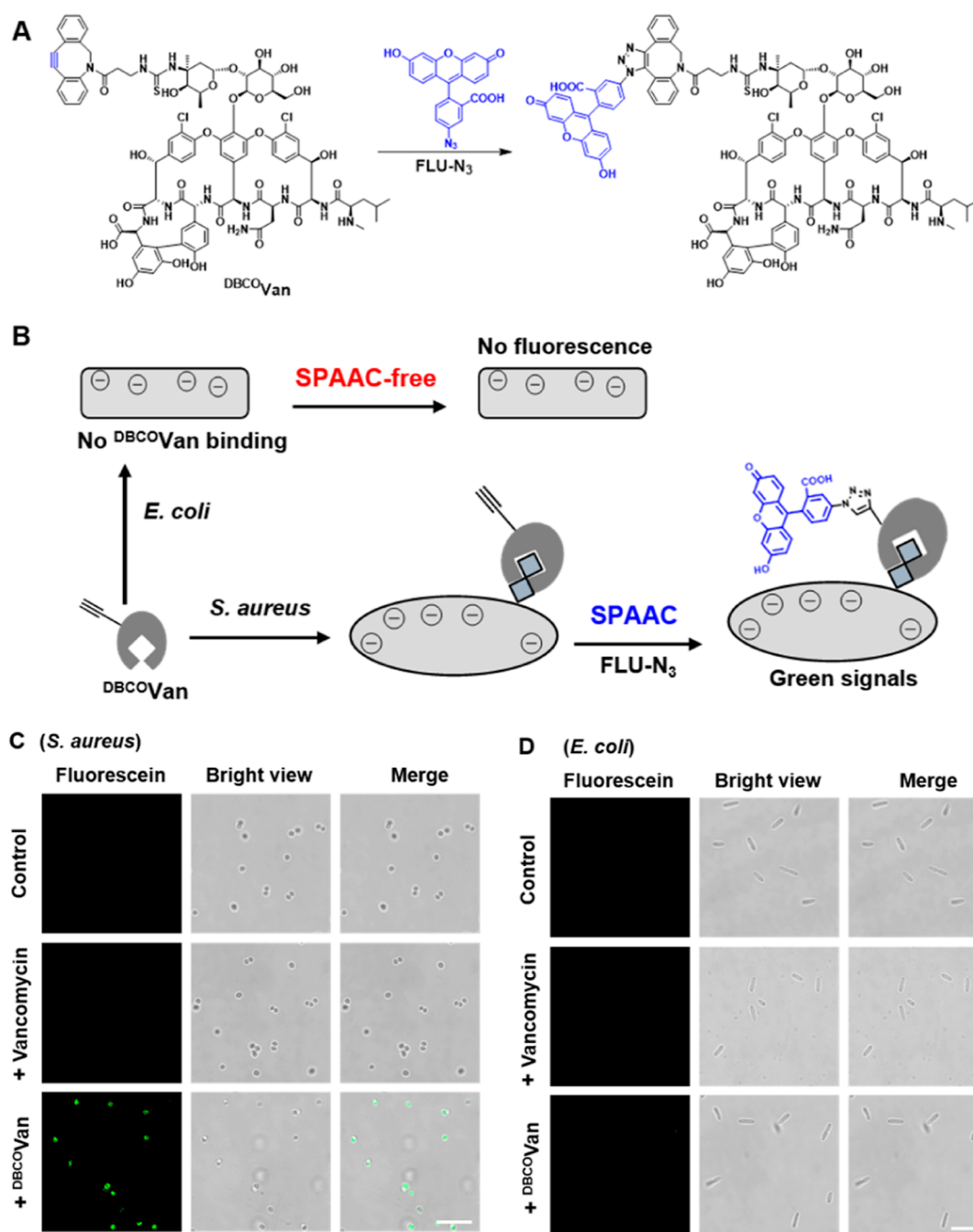**Figure 2.** In vitro SPAAC of ^{DBCO}Van with ^{Az}PolyQa.

^{Az}PolyQa and ^{DBCO}Van alone, peptidoglycan-directed chemical ligation (PGCL) gave rise to synergistic inhibitory effects on Gram-positive *S. aureus* over Gram-negative *Escherichia coli*.

RESULTS AND DISCUSSION

To achieve PGCL, we first synthesized ^{DBCO}Van, which contains a domain of vancomycin with high affinity to D-ala-D-ala in peptidoglycan and a domain of DBCO for bioorthogonal conjugation with azide-containing polycations (Scheme S1, Supporting Information). As cationic materials containing quaternary ammonium have been well-documented to disrupt the bacterial membrane and induce bacterial death,

we thus prepared ^{Az}PolyQa by RAFT[36,37] with 12 repeating units of quaternary ammonium, terminal azide, and a rhodamine entity to trace its binding to the bacterial surface. We examined an in vitro reaction between ^{DBCO}Van with ^{Az}PolyQa. Formation of ^{DBCO}Van-^{Az}PolyQa, the adduct of ^{DBCO}Van and ^{Az}PolyQa, was confirmed by liquid chromatography and mass spectroscopy (Figure S1A,B, Supporting Information), showing the reaction of ^{DBCO}Van with ^{Az}PolyQa in vitro. This is consistent with SPAAC as proposed in Figure 1.

We then examined binding of ^{DBCO}Van to peptidoglycan by treating *S. aureus* with ^{DBCO}Van or vancomycin. Both bacterial

Figure 3. Binding of ^{DBCO}Van to *S. aureus* over E. coli. (A) In vitro SPAAC of ^{DBCO}Van with FLU-N$_3$. (B) Schematic for fluorescence detection of ^{DBCO}Van on peptidoglycan by SPAAC with FLU-N$_3$. (C,D) Fluorescence detection of ^{DBCO}Van bound to *S. aureus* (C) over *E. coli* (D). *S. aureus* and *E. coli* were incubated at 37 °C with vancomycin (25 μM) or ^{DBCO}Van (25 μM) for 1 h and then with FLU-N$_3$ (50 μM) for 1 h. These cells were washed and then visualized by confocal microscopy. Scale bars: 6 μm.

Table 1. MICs of Vancomycin, ^{DBCO}Van and ^{Az}PolyQa against *S. aureus* and *E. coli*. *S. aureus* and *E. coli* Were Incubated with Vancomycin (0.182−186 μg/mL), ^{DBCO}Van (0.221−226 μg/mL), ^{Az}PolyQa (3.9−2000 μg/mL), or ^{DBCO}Van-^{Az}PolyQa (0.977−2000 μg/mL) for 18−24 h, Respectively, and Then Analyzed for MICs

MICs (μg/mL)	Vancomycin	^{DBCO}Van	^{Az}PolyQa	^{DBCO}Van-^{Az}PolyQa
S. aureus	0.725	7.06	31.25	3.91
E. coli	186	>226	62.5	125

samples were washed and then incubated with 5-azidofluorescein (FLU-N$_3$). We observed bright fluorescence in bacteria stained with ^{DBCO}Van/FLU-N$_3$, whereas no fluorescence could be observed on vancomycin$^+$/FLU-N$_3$$^+$ bacteria (Figure 3B,C). As FLU-N$_3$ readily reacts with ^{DBCO}Van by SPAAC (Figures 3A and S2A, Supporting Information), these results showed that ^{DBCO}Van could bind to the cell surface of *S. aureus* and then undergo SPAAC with FLU-N$_3$. *E. coli* prestained with ^{DBCO}Vanc/FLU-N$_3$ exhibited null fluorescence (Figure 3D). As Gram-positive *S. aureus* differs from Gram-negative *E. coli* in exposure of peptidoglycan, the bright fluorescence identified on *S. aureus* over *E. coli* in the presence of ^{DBCO}Van/FLU-N$_3$
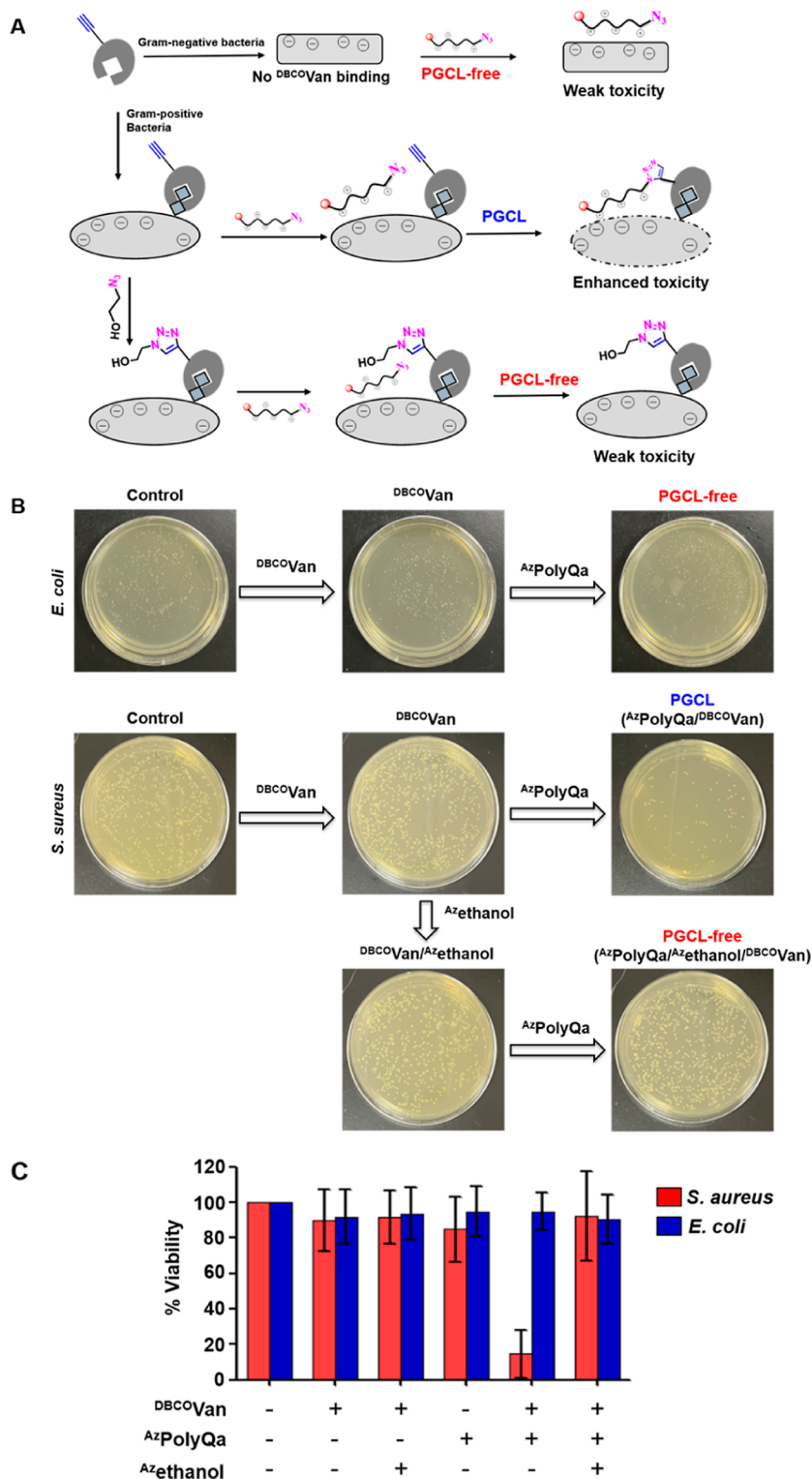
Figure 4. PGCL conferred synergistic bactericidal activity on *S. aureus* but not on *E. coli*. (A) Schematic for PGCL conferred antibacterial activity on Gram-positive bacteria over Gram-negative bacteria. (B) PGCL decreased survival of *S. aureus* while being nontoxic to *E. coli*. *S. aureus* and *E. coli* were treated with [DBCO]Van (3 μM), [Az]PolyQa (15 μg/mL), [DBCO]Van/[Az]PolyQa (PGCL) (3 μM and 15 μg/mL), [DBCO]Van/[Az]ethanol (3 and 3 μM), or [DBCO]Van/[Az]ethanol/[Az]PolyQa (nonPGCL) (3 μM, 3 μM, and 15 μg/mL) at 37 °C for 18 h. The diluted bacterial mixtures were spread on a LB solid agar plate uniformly and then incubated for 18−24 h at 37 °C. (C) Statistic analysis on cytotoxicity of *S. aureus* treated with [DBCO]Van (3 μM), [Az]PolyQa (15 μg/mL), [DBCO]Van/[Az]PolyQa (PGCL) (3 μM and 15 μg/mL), [DBCO]Van/[Az]ethanol (3 and 3 μM), or [DBCO]Van/[Az]ethanol/[Az]PolyQa (nonPGCL) (3 μM, 3 μM, and 15 μg/mL). Error bars represent standard deviations of data for three separate measurements.

showed that [DBCO]Van selectively binds to peptidoglycan, a prerequisite for PGCL. We then treated *S. aureus* and *E. coli*

with [Az]PolyQa. This gave bright fluorescence in *S. aureus*, whereas no fluorescence could be observed on *E. coli* (Figure

S3, Supporting Information), showing that ^{Az}PolyQa preferentially binds *S. aureus*.

Prior to determining whether PGCL could give synergistic antibacterial effects, we assayed the cytotoxicity of ^{DBCO}Van and ^{Az}PolyQa through minimum inhibitory concentrations (MICs). ^{DBCO}Van exerted a pronounced inhibitory effect on *S. aureus* (MICs = 4 μM) over *E. coli* (MICs = 128 μM) (Table 1). Compared to ^{Az}PolyQa, ^{DBCO}Van-^{Az}PolyQa was more toxic to *S. aureus* (MIC: 3.91 μg/mL) but showed less toxicity to *E. coli* (MIC: 125 μg/mL) (Table 1). Albeit much less potent, ^{Az}PolyQa exhibited similar levels of toxicity to *S. aureus* and *E. coli* (MICs: 31.25 and 62.5 μg/mL, respectively) (Table 1). Of note, ^{DBCO}Van, ^{Az}PolyQa, and ^{DBCO}Van-^{Az}PolyQa were largely nontoxic to the mammalian cell line (Figure S4A−C, Supporting Information). Next, PGCL was assessed using *S. aureus* incubated with ^{DBCO}Van and ^{Az}PolyQa. In parallel, non-PGCL was performed using ^{DBCO}Van$^+$ *S. aureus* quenched with 2-azidoethanol (^{Az}ethanol) before exposure to ^{Az}PolyQa as ^{Az}Ethanol effectively reacts with ^{DBCO}Van to consume the DBCO moiety (Figure S2B,C, Supporting Information). The germicidal efficiency on these bacterial samples was evaluated by the surface plating method by calculating the number of colonies. It was revealed that PGCL gave rise to potent antibacterial activity, as evidenced by dramatic decrease in colony forming units relative to non-PGCL, control sample free of treatment, and samples treated with ^{Az}PolyQa, or ^{DBCO}Van either alone or pre-quenched with ^{Az}ethanol (Figure 4A−C). Furthermore, such PGCL-conferred synergistic effects was absent on *E. coli* treated with ^{DBCO}Van and ^{Az}PolyQa (Figure 4B,C). These findings showed the PGCL-mediated potent and selective antibacterial effect for Gram-positive *S. aureus* over *E. coli*.

CONCLUSIONS

Approaches combining microbicides of distinct mechanisms to give synergistic effects show potential to fight drug-resistant bacteria. We herein report peptidoglycan-promoted chemical ligation (PGCL) utilizing DBCO-tagged vancomycin (^{DBCO}Van) and polycationic ^{Az}PolyQa. Binding of ^{DBCO}Van and ^{Az}PolyQa on peptidoglycan triggers PGCL via stain-promoted azide−alkyne cycloaddition. Compared to ^{Az}PolyQa and ^{DBCO}Van, each alone fails at killing bacteria at low doses, and PGCL yields synergistic bactericidal effects on Gram-positive *S. aureus* over Gram-negative *E. coli*. These findings suggest the feasibility of peptidoglycan-confined chemical ligation such as PGCL for selective ablation of Gram-positive bacteria, indicating an alternative perspective against pathogens.

ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c06964.

> Synthesis of ^{DBCO}Van, ^{Az}PolyQa, and ^{DBCO}Van-^{Az}PolyQa; in vitro SPAAC of ^{DBCO}Van and FLU-N$_3$, ^{DBCO}Van, and ^{Az}ethanol; determination of antimicrobial activity (MIC assay); and determination of bactericidal activity through chemical ligation of antibiotics on the bacterial membrane (PDF)

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Notes

The authors declare no competing financial interest.

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