**Never Trust a Phage Bearing Gifts: Nitroxide Enhances Phage-Ceftazidime Synergy**

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**Background and aims.** The multi-drug-resistant bacteria, *Pseudomonas aeruginosa* (*P. aeruginosa*), plagues patients globally with biofilm infections which greatly tolerate antibiotics. (1) Bacteriophage (phage) therapy is a promising alternative strategy to combat antimicrobial resistance, and combinatory use with antibiotics can synergise (phage-antibiotic synergy, PAS) to overcome bacterial biofilm infections. (2) This project developed a phage-drug conjugate formulation in which phage “nanobots” targeted the delivery of the therapeutic payload at *P. aeruginosa* biofilms.

**Methods.** The antibiotic, ceftazidime (CAZ), and antibiofilm agent, 4-amino-TEMPO (nitroxide) were conjugated to Pae7 phages via a hydrolysable imine bond to the linker, POEGA-*b*-PVBA, prepared by RAFT polymerisation. Phages dually served as an antibacterial agent and drug delivery system, specifically delivering the therapeutic cargo to preformed, *P. aeruginosa* biofilms for 24-hour treatment. Biofilm biomass dispersal was quantified with crystal violet staining and bacterial cell viability was determined by colony forming unit (CFU) counting.

**Results.** POEGA-*b*-PVBA exhibited stimuli-responsiveness with the controlled release of antibacterial agents in mildly acidic conditions like that of biofilms. At preformed *P. aeruginosa* biofilm sites, the CAZ (8 μg mL-1) and Pae7 dual-therapy eradicated planktonic bacteria, dispersed up to 97% of the biofilm biomass and significantly reduced biofilm cell counts by up to 3.63 log. Triple-therapy with nitroxide enhanced this PAS phenomenon; using 2 μg mL-1 of CAZ, 94% of the biofilm biomass and 3.38 log of biofilm cells were cleared.

**Conclusion/Discussion.** This single-packaged phage-drug conjugate formulation utilises a functional and stimuli-responsive linker to facilitate selective payload release at biofilm infection sites. Correct selection of drugs and phages for pathogen-specific delivery can maximise local therapeutic deposition and efficacy, minimise off-target effects, and the exertion of PAS and controlled release approach can reduce antibiotic load in patients while overcoming antibiotic-resistant infections.

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