

Synergistic Action of Bacteriophage and Vancomycin in a Co-delivery hydrogel for Localized Treatment of Fracture-Related Infection caused by Methicillin-resistant *Staphylococcus aureus*.

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INTRODUCTION: Bacteriophages are increasingly being considered as alternative and adjunctive therapy for difficult to treat bone and joint infections. However, current administration protocols involve prolonged retention of a percutaneous draining tube with potential risk of developing superinfection. In this study, we applied a cocktail of *in vitro* evolved biofilm-targeting phages for Methicillin-resistant *Staphylococcus aureus* (MRSA) in a hydrogel platform co-delivering vancomycin. *In vitro* synergy and antibiofilm activity were assessed and a subsequent *in vivo* study was performed in a mouse bone infection model with MRSA.

METHODS: A cocktail of environmentally sourced bacteriophages were subjected to an *in vitro* evolution experiment with enhanced MRSA3 biofilm killing ability being selection criteria for each cycle. After 30 cycles, two phages (MRSA-R14 and COL-R23) with improved antibiofilm activity were identified and then tested in combination with vancomycin and a carboxymethylcellulose (CMC) hydrogel *in vitro* and *in vivo*. MRSA3 bacterial biofilms were formed on sterile 4 mm sintered porous glass beads at 37 °C for 24 h. Biofilms were exposed to i) phage cocktail (10⁷ PFU/ml), ii) vancomycin at concentrations of 0.5, 1, 10 and 100 times the MIC, or iii) combination of phage cocktail and vancomycin. Recovered biofilm cells, were quantified by colony counting. The stability and release profiles of phage cocktail and vancomycin in co-delivery hydrogel were assessed *in vitro* for 8 days and 72 hrs, respectively, and subsequently tested in the treatment of 5-day-old MRSA3 infection of a femoral plate osteotomy in mice.

RESULTS SECTION: *In vitro*: The cocktail of evolved phages (10⁷ PFU/ml, 1:1) combined with 0.5 MIC vancomycin achieved 99.72% reduction in MRSA3 biofilm *in vitro* compared to the growth control. This combination was stable in the co-delivery hydrogel over 8 days. The release profile showed that 57% of phages and 80% of vancomycin were released after 72 hrs, which was identical to the performance for gels loaded with phage or antibiotic alone. In the *in vivo* study, the bacterial load from animals that received co-delivery hydrogel and systemic vancomycin was significantly reduced compared to controls, animals that received systemic vancomycin and animals that received co-delivery hydrogel alone (*p*<0.05).

DISCUSSION: Our study demonstrates the potential of using evolved phages in combination with vancomycin and hydrogel delivery systems for the treatment of MRSA-related infections. *In vitro* evolution enhanced biofilm killing significantly, as did partnering with vancomycin. Hydrogel delivery system also simplified delivery, removing the need for repeated instillations.

SIGNIFICANCE/CLINICAL RELEVANCE: As antibiotic resistance continues to reduce efficacy of conventional antibiotic therapy for bone and joint infections, new therapies are required. Phage therapy has shown promise in recent clinical reports; however, we show improved efficacy may be achieved by adapting phage to target biofilm and by partnering with synergistic antibiotic partners. Finally, delivery in hydrogel mode simplifies administration, and was found to improve bacterial reduction in an orthotopic mouse model.