biofilms | Pseudomonas aeruginosa | Staphylococcus aureus | ex vivo models | phage/antibiotic combinations

Efficacy against dual-species biofilms using phage-antibiotics combinations is independent of the biofilm model

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Pseudomonas aeruginosa and *Staphylococcus aureus* are opportunistic pathogens commonly found in polymicrobial infections, namely in wounds and in respiratory tract infections. Both organisms frequently cause chronic biofilm infections and due to their antibiotic tolerance are very challenging to control. We have previously shown that the combined treatments of *Pseudomonas* phage EPA1 and gentamicin have increased anti-biofilm activity against mono and dual-species biofilms formed in microtiter plates.

Here, we developed an innovative approach to study the efficacy of phage-antibiotic combinations in two *in vivo*-like models: a three-dimensional lung epithelial model that mimics aspects of the parental tissue and an artificial wound model. The efficacy of single, simultaneous and sequential treatments were compared. In the lung model, the sequential treatment of phages and gentamicin resulted in *P. aeruginosa* biofilm eradication. In artificial dermis, sequential treatment was also the treatment where higher reductions of culturable cells was observed in dual-species biofilms. Globally, our data suggests that the sequential phage treatment causes an adjuvant effect by lowering the MIC value of the phage-surviving population.

LDH test showed that this sequential application of phages and antibiotics is not cytotoxic to lung cells. In addition, we observed that on the lung model the 3-D cell integrity was not affected by sequential treatments.

We also demonstrated that the order in which phages and antibiotics are applied lead to different efficacy outcomes, showing that in clinical practice the timing to apply antibiotics will be very crucial for the success of treatment.

The sequential application of phages and ciprofloxacin was shown to be safe and very efficient against dual-species biofilms formed in different models simulating different types of infection and opening new perspectives for their clinical application.