Phage-Antibiotic Synergy for the Treatment of Biofilm-related Infections on Orthopedic Implants

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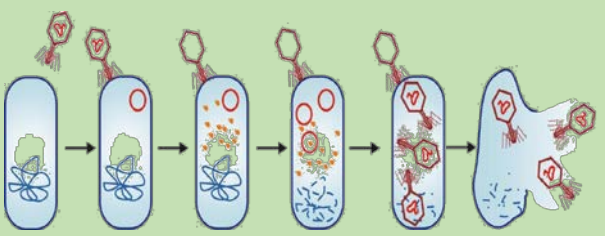
Introduction

Bacterial infections are often **complicated** due to the **formation of biofilms**, especially in the setting of medical implantable devices such as **orthopedic implants**.

These (poly)microbial micro-communities, which are embedded in a self-produced polymeric matrix, are known to be **much more resistant** when **compared to their planktonic counterparts** due to this physical barrier as well as the **metabolic shift** these bacteria have undergone during biofilm maturation**.**

Bacteriohages, which **infect and kill only bacterial cells** in a **very host specific** manner could become even more attractive due to their **potential enzymatic effects on biofilm matrix components,** which are causing difficult-to-treat infections such as seen in the orthopedic setting.



Objectives

Devising a **novel treatment modality** for **biofilm related infections on orthopedic** implants by investigating and exploiting the **synergy** between de novo isolated **bacteriophages and routinely used antibiotics**, focusing on *P. aeruginosa* which is involved in rare but severe orthopedic infections.

Methods

79 phage clones active against *P.aeruginosa* were de novo **isolated** from a wide variety of environmental sources including **hospital sewage waters**, water samples from **parks and lakes**, **wound compresses** from patients having a recurrent infection and even **Human Breast Milk (HBM) samples**. **Phages** were subsequently **purified, propagated and characterized** (plaque morphology, host range & genetic sequencing).

Of this broad spectrum phage bank, **20 phages** with broad host range and **lytic activity** against *P.aeruginosa* PAO1 were identified and **tested on PAO1 biofilms**.

Five phages showing the highest antibiofilm activity, have subsequently been **tested in combination with ciprofloxacin, meropenem and ceftazidime** for their effects on CFU counts, biomass and metabolic activity (Omnilog Biotyper). Scanning electron microscopy was performed for PAO1 biofilms grown on titanium coupons (mimicking the implant material) to gain more insights on biofilms structure after different treatment protocols.

Genetic sequencing identified the phages presented here today as a **Yuavirus** (Siphoviridae) being phage 2, a **Pbunavirus** (Myoviriday) being phage 3 and a **Bruynoghevirus** (Podoviridae) being phage 30.

Results

Biofilms are known to be **100-10000 times more tolerant towards antibiotics** than their planktonic counterparts. However, when using **antibiotics at their MIC** in **combination with bacteriophages**, **significant reductions** are seen for **both CFU** counts on selective plates as for **biomass** evaluations (figure 1). When increasing antibiotic concentrations to 10xMIC, an even higher decrease on biomass & viable population was observed (not shown). These findings have been **confirmed with metabolic assays using the Omnilog Biotyper** (figure 2).

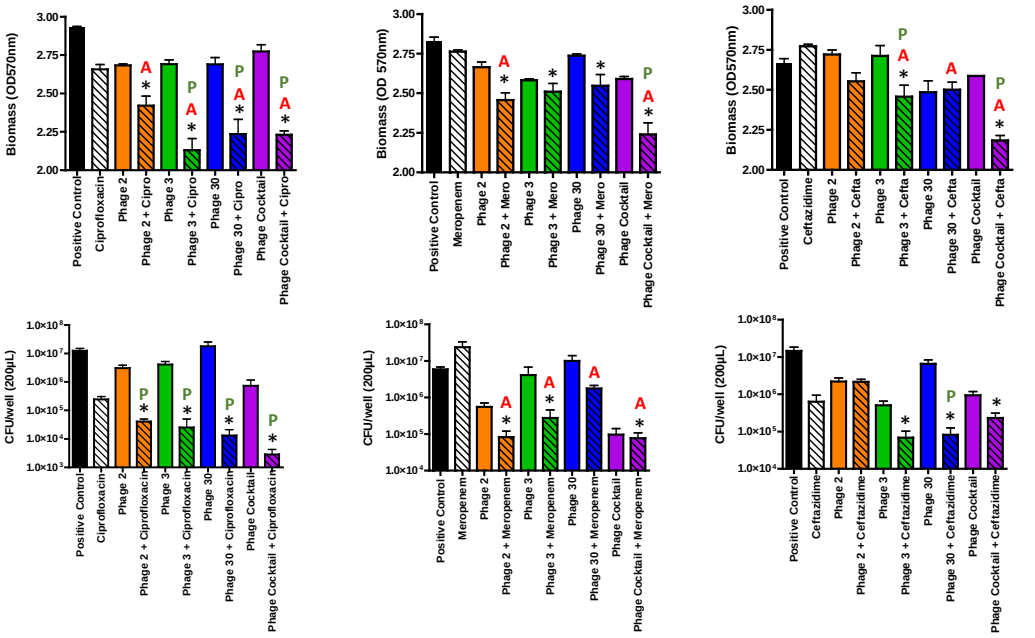


Figure 1. Biomass evaluation (upper) and CFU count on selective plates (down) for the application of phages (10^9 PFU/mL) with or without the presence of antibiotics at 1x the MIC (ciprofloxacin = left, meropenem = middle & ceftazidime = right). Statistical analysis: star indicates significant reduction compared to positive control, capital **A** indicates significant reduction compared to antibiotic alone and capital **P** indicates reduction compared to phage alone ($p<0.05$). N = 3 & n = 60

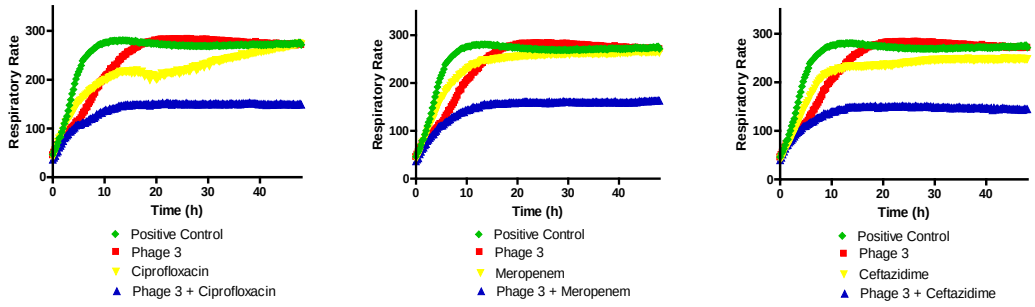
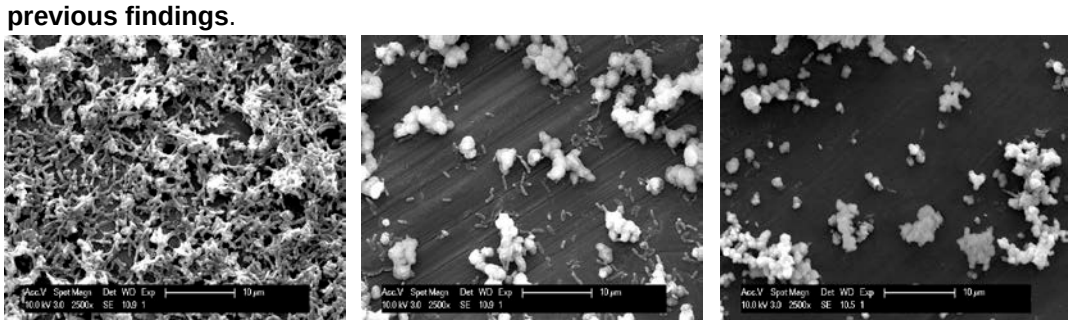


Figure 2. Metabolic assay measuring respiratory rate of PAO1 biofilms after phage (10^9 PFU/mL) and/or antibiotic (10xMIC) application. Left indicates applications with ciprofloxacin, middle for meropenem and right for ceftazidime. N=2, n=72. Results indicate a significant reduction in respiratory rate of the biofilm when both phage and antibiotic are added as a combined treatment to PAO1 biofilms.

Scanning electron microscopy for the application of phage 30 in combination with ciprofloxacin (10xMIC) on PAO1 biofilms, grown on titanium coupons **confirmed previous findings**.



Untreated PAO1 biofilm | **24h Phage + Ciprofloxacin** | **48h Phage + Ciprofloxacin**

Conclusion

A **combination of phages and antibiotics is capable of reducing CFU counts**, thereby **reducing biofilm respiratory rate** as seen with Omnilog assays, **as well as biomass of PAO1 biofilms** grown **in-vitro and on titanium coupons** more efficiently than each type of agent alone. Further research to try and identify the most optimal conditions of exposure, i.e. combined or sequential application is currently being performed. **Phage coating possibilities are also being explored**.

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