Comparison of Three Aqueous Aerosol Inhalation Devices for Delivering Anti-tuberculosis Bacteriophage D29

Nicholas Carrigy
Bacteriophage: An Alternative to Antibiotics

- Antibiotic-resistance is a threat to global health
  - 480,000 new multidrug-resistant tuberculosis cases in 2015, 9.5% further classified as extensively drug-resistant [1]
  - Few new antibiotics are being developed

- Bacteriophage (phage) are an alternative
  - They can infect antibiotic-resistant bacteria

What is a Phage? – A Virus that Infects Bacteria

![Phage Diagram](https://en.wikipedia.org/wiki/Bacteriophage)

**Phage**
- DNA-filled capsid
- Sheath through which DNA is injected to bacteria
- Tail fibers with bacterial wall receptors
- Base plate

**Lytic Cycle**
- Uninfected cell
- Phage adsorption
- Cell lysis
- Viral release

Image on left adapted from: https://en.wikipedia.org/wiki/Bacteriophage
**Phage Therapy**

**Why phage therapy?**
- Lytic phage can infect antibiotic-resistant bacteria
- High specificity, not harmful to beneficial bacteria
- Few if any side effects; phage are everywhere

**Will it work?**
- Human phage therapy done in Eastern Europe
  - Phage cocktails available over-the-counter
  - Efficacy reports are generally positive
- Phage used in food production and compassionate care in the USA
  - Human clinical trials ongoing including PhagoBurn here in France and AmpliPhi in the USA
- Success of phage aerosol delivery to mice to clear antibiotic-resistant BCC lung infections requires that many active phage reach the lungs relative to the bacterial count [2]

Why Test Phage D29?

- Phage D29 infects *M. tuberculosis* [3]
  - It also infects *M. smegmatis*, which is biosafety level 1
  - Its genome has been sequenced, and it has well-established amplification and assay protocols

TEM of phage D29, which lyses *M. tuberculosis*

Plaque assay determines number of active phage in a sample

Image on right from: phagesdb.org/workflow
Phage Deactivation due to Aerosolization

Tested inhalation devices

1) Vibrating Mesh Nebulizer

2) Jet Nebulizer

3) Soft Mist Inhaler

- Deactivation = (1 – output titer / input titer) * 100%
  - Input titer = # active phage in saline phage preparation input to each inhalation device
  - Output titer = # active phage captured on filter after aerosolization

Images from: https://www.inspiration-medical.de/Bilder/Aerogen%20Solo%20Vernebler%20ex%20Aeroneb.jpg
https://online.ebos.co.nz/images/product/22143069%20-%20BOY%20OSX.jpg
http://d3hjf51r9j54j7.cloudfront.net/wp-content/uploads/sites/5/2008/01/spiriva_respmatw_image1_3.jpg
Difference in Phage Deactivation between Devices

<table>
<thead>
<tr>
<th>Inhalation Device</th>
<th>Deactivation (%) *</th>
<th>Active Phage Delivery Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jet Nebulizer</td>
<td>99.981 ± 0.005</td>
<td>7.1x10^4 ± 1.7x10^4 pfu/min</td>
</tr>
<tr>
<td>Vibrating Mesh Nebulizer</td>
<td>60 ± 11</td>
<td>3.3x10^8 ± 0.8x10^8 pfu/min</td>
</tr>
<tr>
<td>Soft Mist Inhaler</td>
<td>72 ± 14</td>
<td>4.6x10^6 ± 2.0x10^6 pfu/dose</td>
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</tbody>
</table>

* < 90% deactivation is acceptable

- Vibrating mesh nebulizer delivered active phage D29 ~5000 times faster than the jet nebulizer
- A single 11.6 ± 1.6 µL ex-actuator dose from the soft mist inhaler delivered about as many active phage D29 as 1 hour of delivery with the jet nebulizer, which would require about 10 mL of formulation
Reason for Titer Reduction with the Jet Nebulizer

- Likely stress during baffle impaction and renebulization
- Previously reported to deactivate liposomes & large molecules [4]

99% of aerosol was renebulized in each cycle

- Equivalent of entire 8 mL recirculated every 30 seconds, in agreement with literature [5]
- Large cumulative stress on phage
Vibrating Mesh Nebulizer and Soft Mist Inhaler

- Droplet production mechanisms with the vibrating mesh nebulizer (left) and soft mist inhaler (right) were relatively unharmful to phage D29
Conclusions

- Pulmonary delivery of anti-tuberculosis phage D29 at high titers requires a prudent choice of inhalation device
  - Titer reduction is inhalation device- and phage strain-dependent

- Jet nebulizer
  - Not recommended for phage therapy with D29 - substantial titer reduction

- Vibrating mesh nebulizer
  - Recommended for animal studies - small titer reduction, high active phage delivery rate

- Soft mist inhaler
  - Recommended for self-administration - small titer reduction, pocket-sized, multidose

- Aerosol delivery of phage is feasible, and promising
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