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

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Introduction

- Antibiotic-resistance
- Phage therapy is an alternative [1,2]
- Need many phage in lungs relative to bacteria [3]
- Inhalation device must not deactivate the phage

Materials and Methods

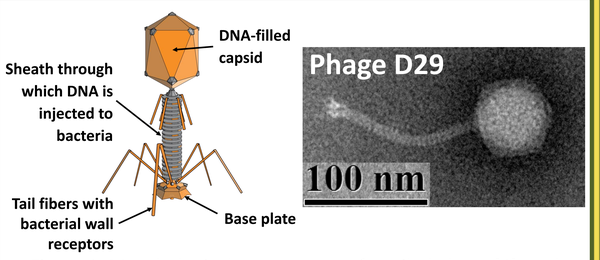

Figure 1: Morphological components of a tailed phage [4], and TEM of phage D29, which lyses *M. tuberculosis*.

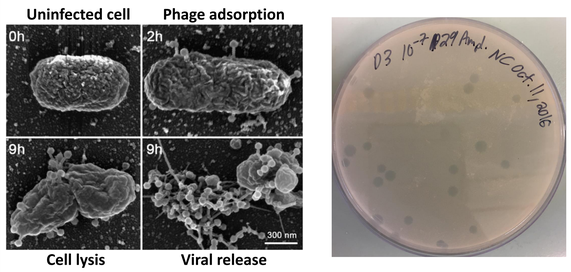

Figure 2: Lytic cycle [5], and *M. smegmatis* plaque assay plate.

Aerogen Solo vibrating mesh nebulizer

Pari LC Sprint jet nebulizer

Respimat soft mist inhaler

Suregard aerosol collection filter

Figure 3: Tested inhalation devices & collection filter [6-9].

Results and Discussion

Table 1: Phage D29 deactivation due to aerosolization and rate active phage exit the respective devices.

Inhalation Device	Deactivation (%) *	Active Phage Delivery Rate
Jet Nebulizer	99.981 ± 0.005	$7.1x10^4 \pm 1.7x10^4$ pfu/min
Vibrating Mesh Nebulizer	60 ± 11	$3.3x10^8 \pm 0.8x10^8$ pfu/min
Soft Mist Inhaler	72 ± 14	$4.6x10^6 \pm 2.0x10^6$ pfu/dose

* < 90% deactivation is acceptable

Jet Nebulizer

Deactivated 99.98% of D29

Reason: likely stress during baffle impaction and renebulization

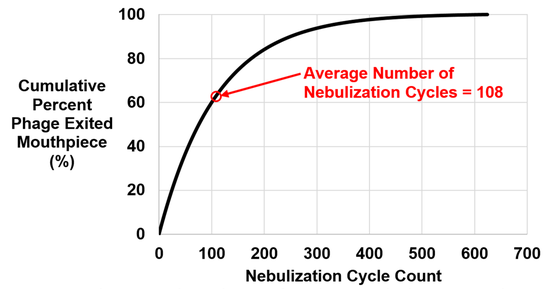

Figure 4: Mathematical prediction for phage nebulization cycle count with the jet nebulizer.

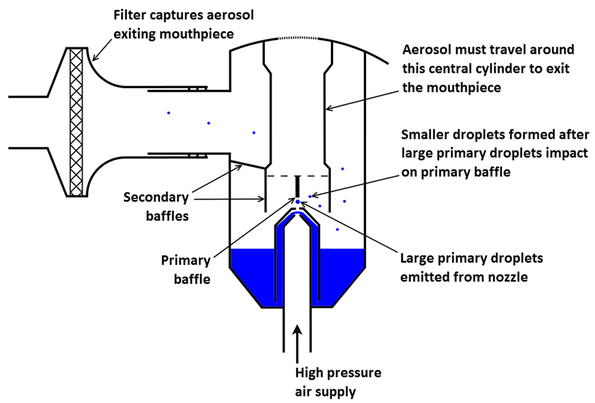

Figure 5: Droplet production with the jet nebulizer.

Vibrating Mesh Nebulizer

Relatively unharmful to D29

Delivers active phage D29 ~5000 times faster than the jet nebulizer

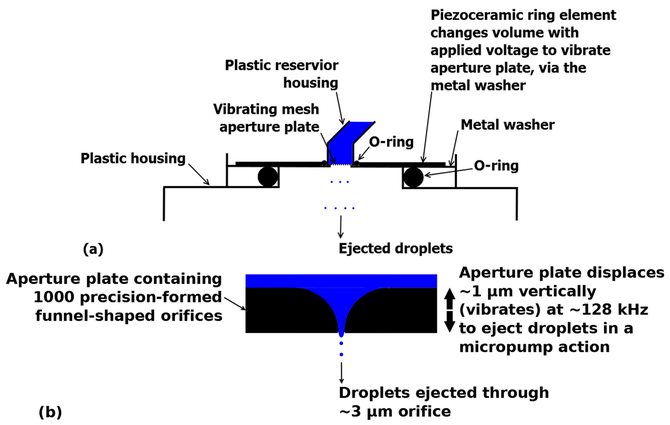

Figure 6: Vibrating mesh nebulizer droplet production mechanism: (a) the structure surrounding the aperture plate, (b) close-up of a funnel-shaped orifice in the aperture plate illustrating the micropump action, which ejects droplets.

Soft Mist Inhaler

Relatively unharmful to D29

A single 11.6 ± 1.6 µL dose delivers as much active phage as 1 hour of delivery with the jet nebulizer

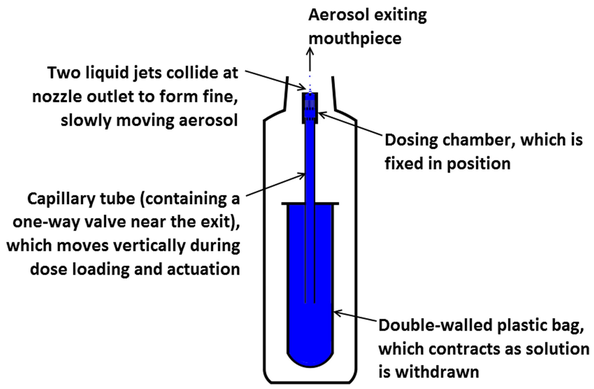

Figure 7: Schematic of the soft mist inhaler. The shear and pressurization was relatively unharmful to D29.

Conclusions

- Ensure your inhalation device does not deactivate the phage before starting pulmonary experiments
- Titer reduction is inhalation device- and phage strain-dependent
- Jet nebulization can deactivate D29; deactivation is likely due to stresses during baffle impaction and renebulization
- Other phage readily survive jet nebulization [10]
- Vibrating mesh nebulizer is a good choice for starting animal studies with D29
- Soft mist inhaler may be useful for self-administration, being pocket-sized and multidose
- Phage therapy using inhalation devices is feasible, and promising

References

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