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

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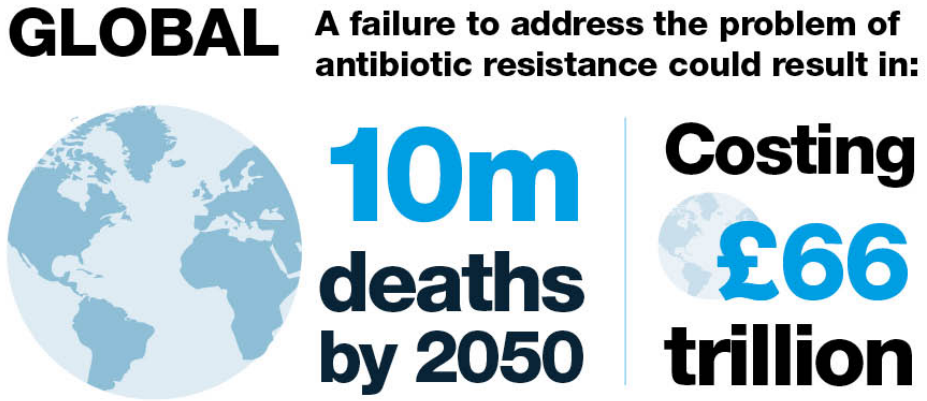
MECHANICAL ENGINEERING

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

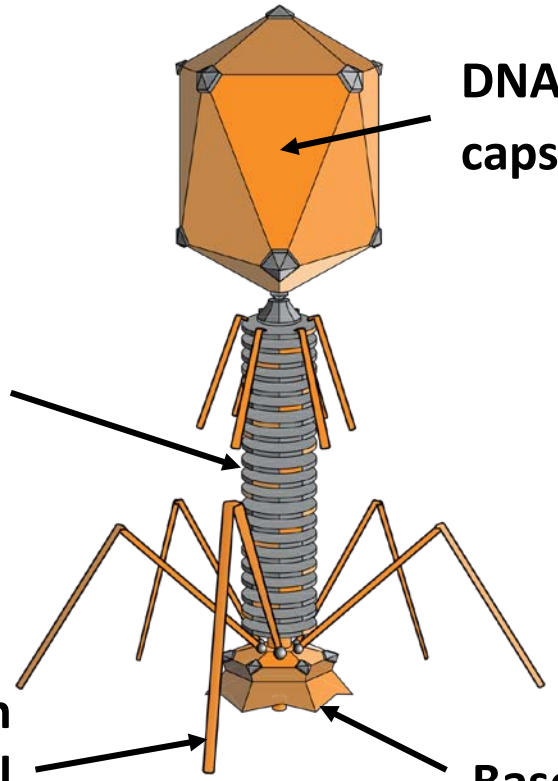
- Bacteriophage (phage) are an alternative
  - They can infect antibiotic-resistant bacteria



[1] WHO. Multidrug-resistant tuberculosis (MDR-TB) 2016 Update. From: http://www.who.int/tb/challenges/mdr/mdr_tb_factsheet.pdf
Images from: https://cdn.ibdnewstoday.com/wp-content/uploads/2015/07/shutterstock_103632251.jpg
https://www.gov.uk/government/publications/health-matters-antimicrobial-resistance/health-matters-antimicrobial-resistance

What is a Phage? – A Virus that Infects Bacteria

Phage



Lytic Cycle

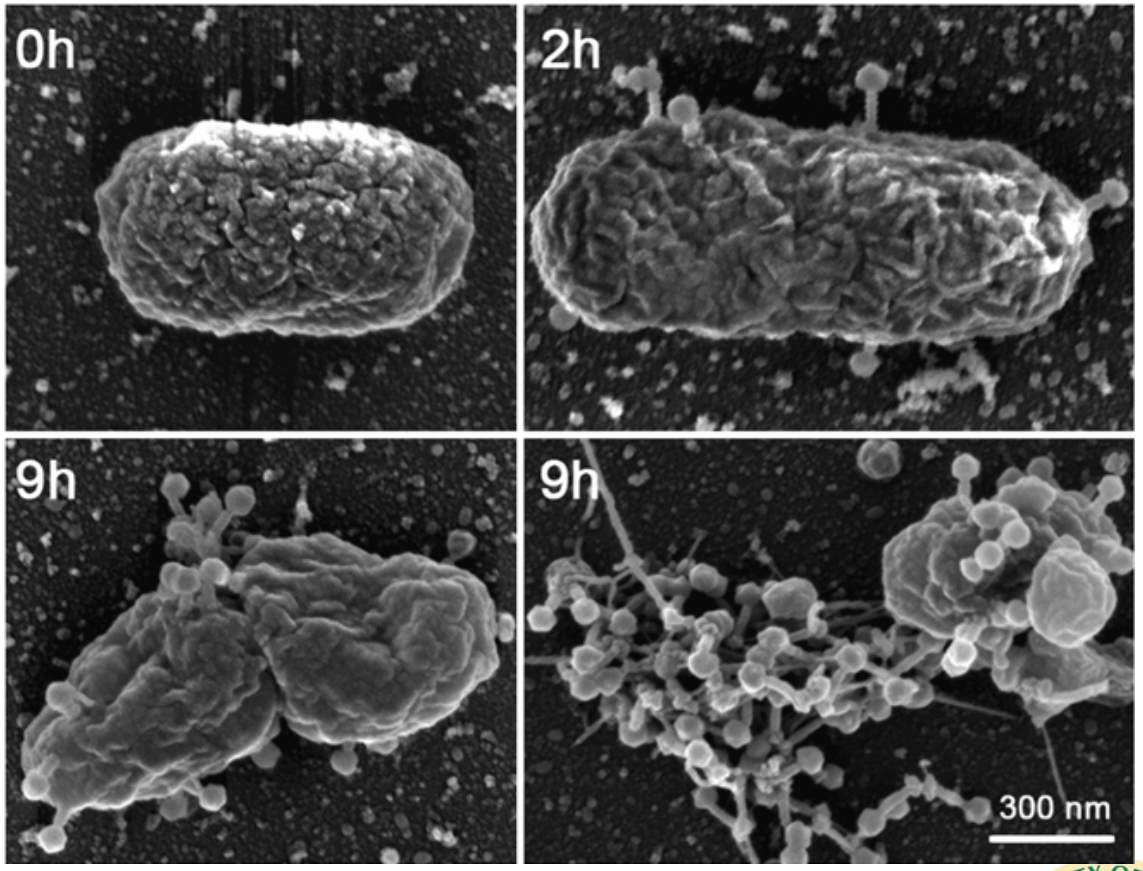


Image on left adapted from: https://en.wikipedia.org/wiki/Bacteriophage

Image on right adapted from: Sabehi G. *et al.* (2012) PNAS 109(6):2037-2042.

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?

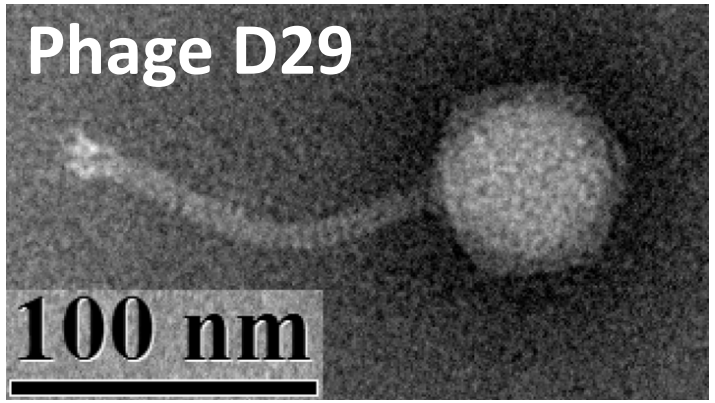
- Human phage therapy done in Eastern Europe
  - Phage cocktails available over-the-counter
  - Efficacy reports are generally positive

- 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]

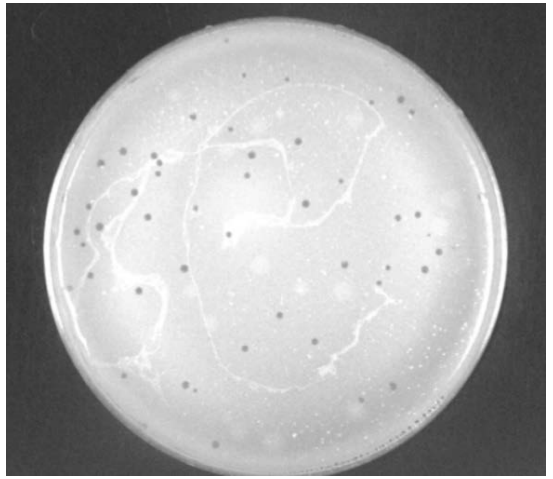
[2] Semler DD *et al.* (2014) Antimicrob Agents Chemother 58(7):4005-4013.
Images from: https://www.alexbetts.info/single-post/2015/10/30/Clinical-Trials-and-Phage-Therapy

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

[3] Froman S *et al.* (1954) Am J Public Health Nations Health 44(10): 1326-1333.

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%20SX.jpg
http://d3hjf51r9j54j7.cloudfront.net/wp-content/uploads/sites/5/2008/01/spiriva_respimatw_image1_3.jpg

Difference in Phage Deactivation between Devices

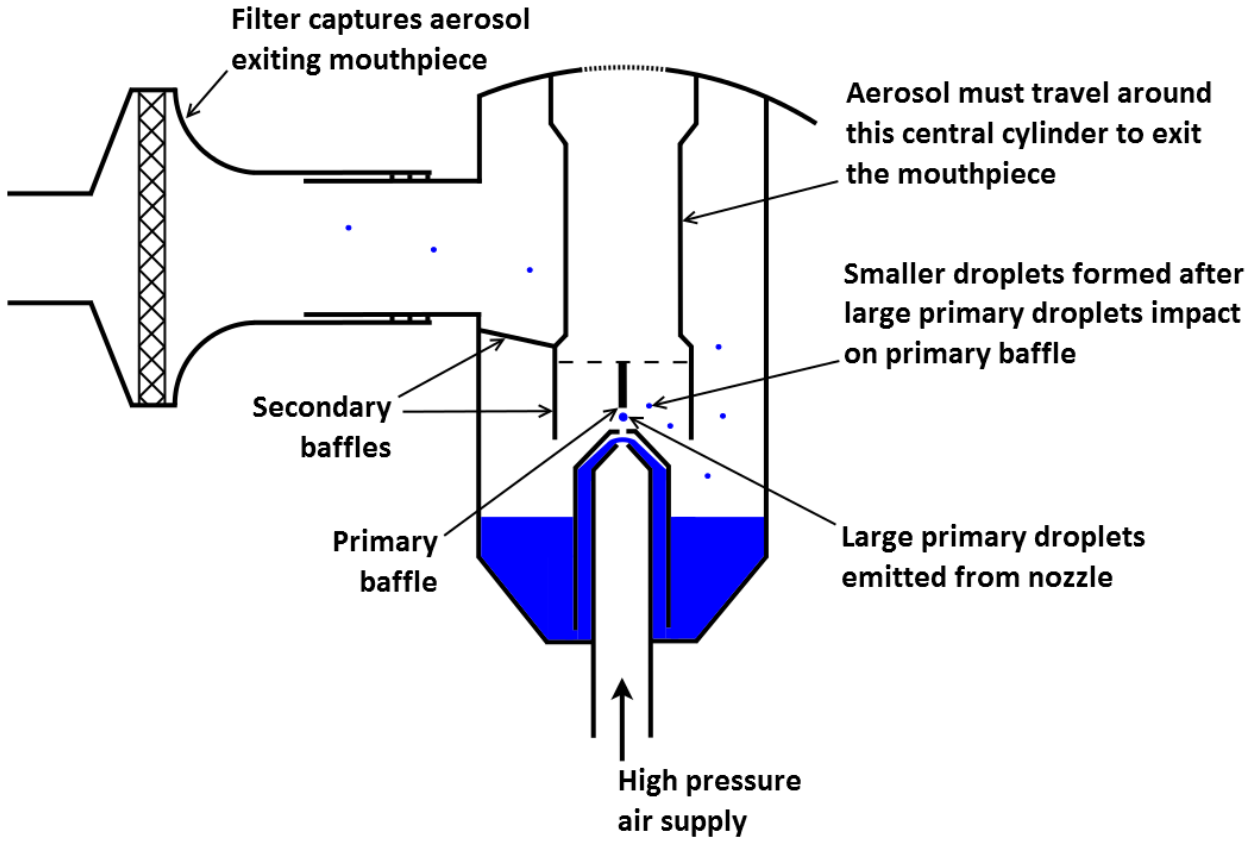
Inhalation Device	Deactivation (%) *	Active Phage Delivery Rate
Jet Nebulizer	99.981 ± 0.005	$7.1 \times 10^4 \pm 1.7 \times 10^4$ pfu/min
Vibrating Mesh Nebulizer	60 ± 11	$3.3 \times 10^8 \pm 0.8 \times 10^8$ pfu/min
Soft Mist Inhaler	72 ± 14	$4.6 \times 10^6 \pm 2.0 \times 10^6$ pfu/dose

* < 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

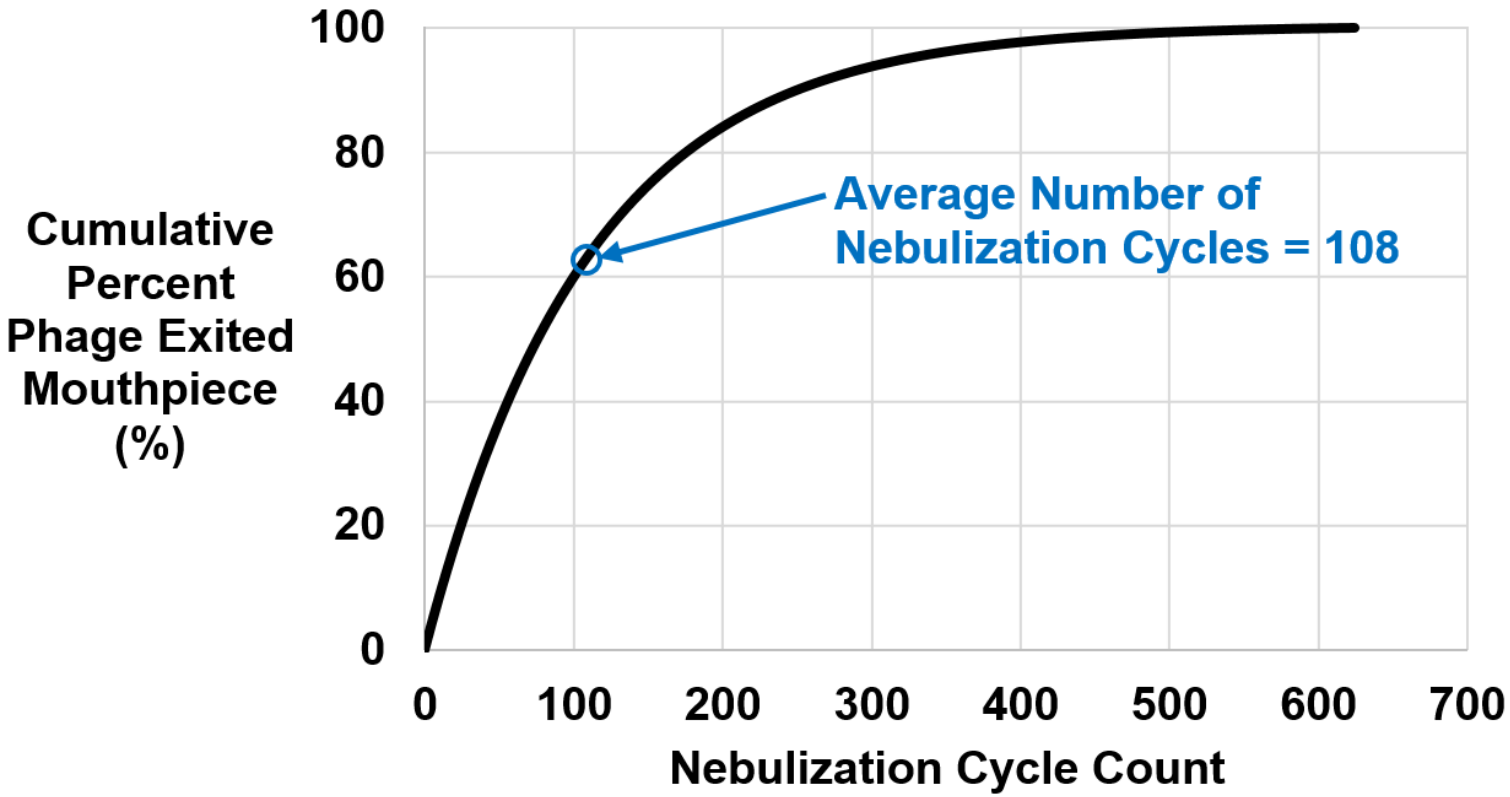
- Likely stress during baffle impaction and renebulization
  - Previously reported to deactivate liposomes & large molecules [4]



[4] Lentz YK *et al.* (2005) J Aerosol Sci 36(8): 973-990.

Renebulization with the Jet Nebulizer – A Mathematical Model

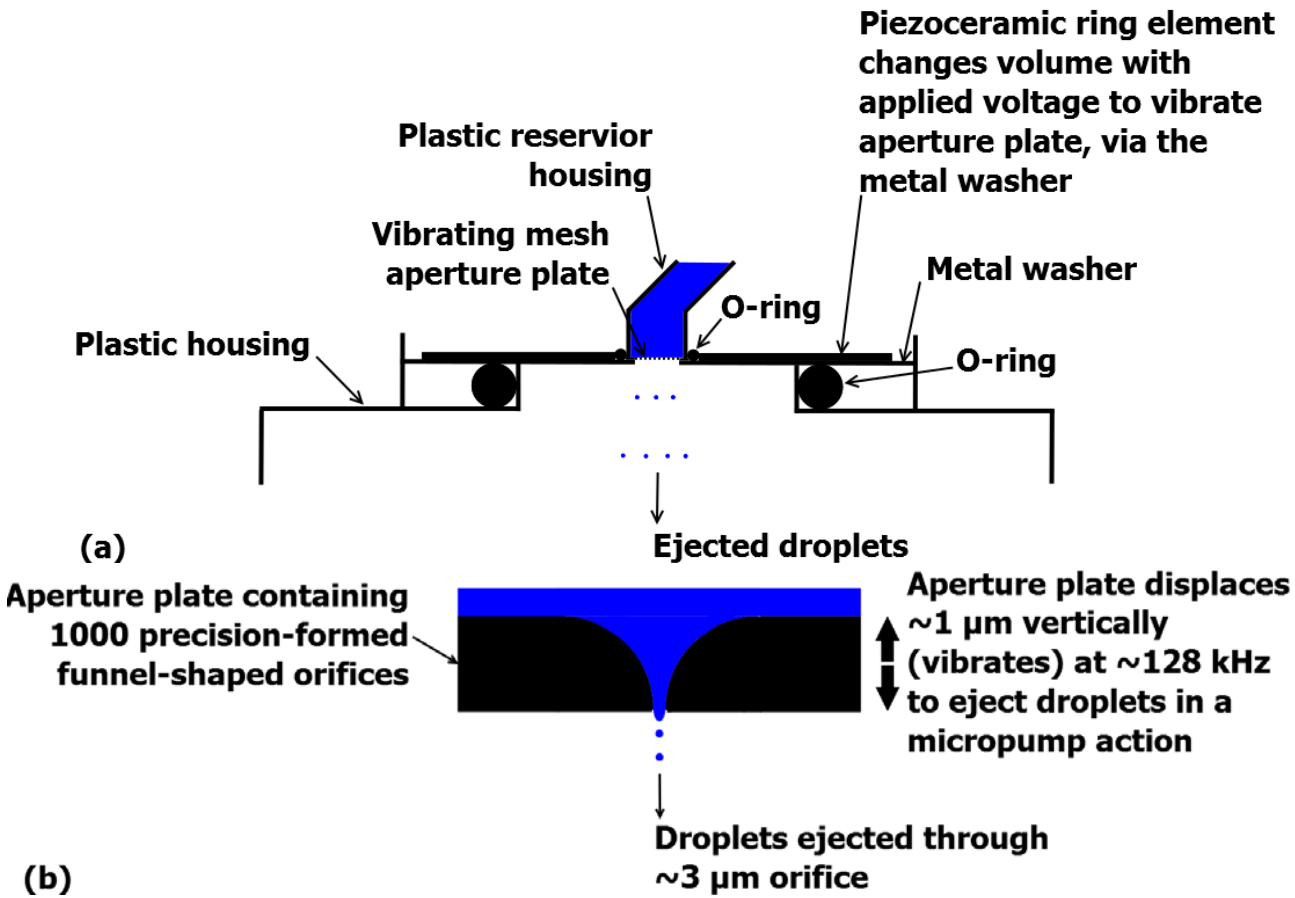
- 99% of aerosol was renebulized in each cycle
  - Equivalent of entire 8 mL recirculated every 30 seconds, in agreement with literature [5]
  - Large cumulative stress on phage

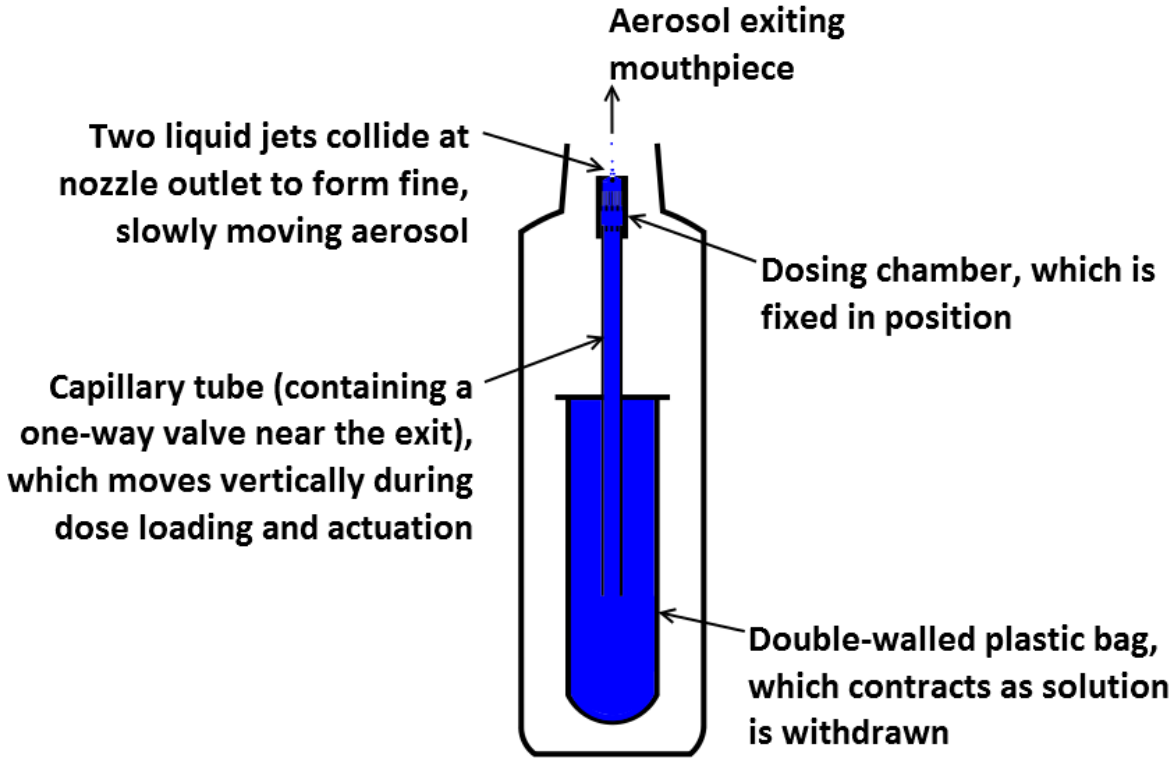


[5] May KR. (1973) J Aerosol Sci. 4(3):235-243.

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

Acknowledgements

University of Alberta

Prof. Reinhard Vehring
Prof. Warren H. Finlay
Prof. Dominic Sauvageau
Melissa Harrison

University of Sydney

Prof. Hak-Kim Chan
Prof. Warwick J. Britton
Dr. Rachel Y. Chang
Dr. Sharon S.Y. Leung

University of Pittsburgh

Prof. Graham F. Hatfull
Prof. Welkin H. Pope
Zaritza Petrova

Funding

Australian Research Council Discovery Project DP150103953

Natural Sciences and Engineering Research Council of Canada

Alberta Innovates Technology Futures

University of Alberta Scholarships & Awards