Altering the size distribution of influenza virion populations

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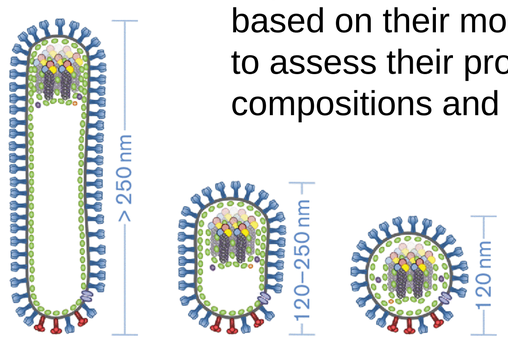
1. Filamentous influenza

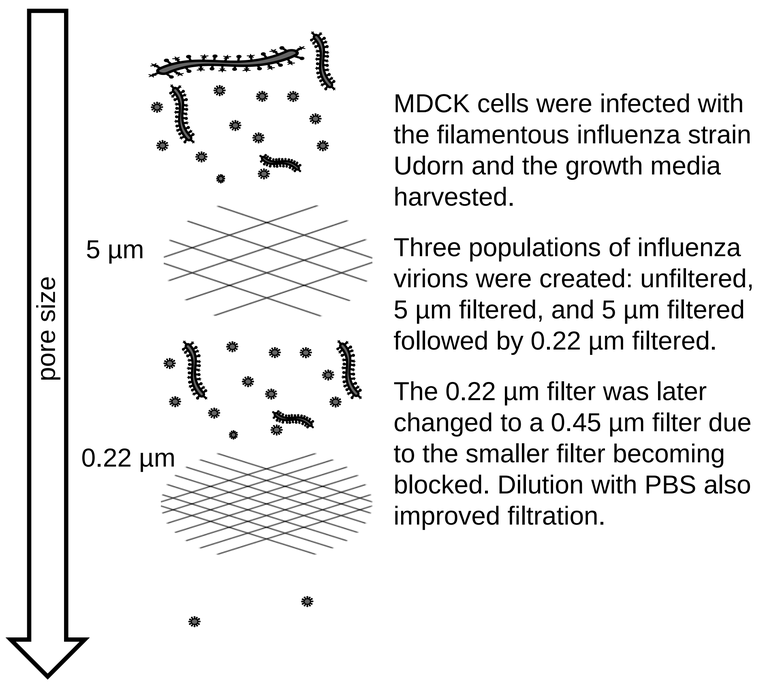
- Clinical and veterinary isolates of influenza virus produce both spherical and filamentous virions[1].
- Most laboratory-adapted strains of influenza produce only spherical virions.
- Despite being discovered in 1946, the role of filaments is not yet known. Basic tools to study filaments are lacking, including the ability to separate them from other virion morphologies.
  - This project aimed to separate populations of influenza virions based on their morphology and to assess their protein compositions and infectivity.



Morphologies of influenza virions. Filamentous, bacilliform and spherical [2].

2. Methods

- During a six week summer project, samples of influenza virions with varying morphological distributions were prepared by filtration.
- Filtered virions were counted and measured by negative-stain TEM.
- Filtration reduced the number of long filamentous virions observed.

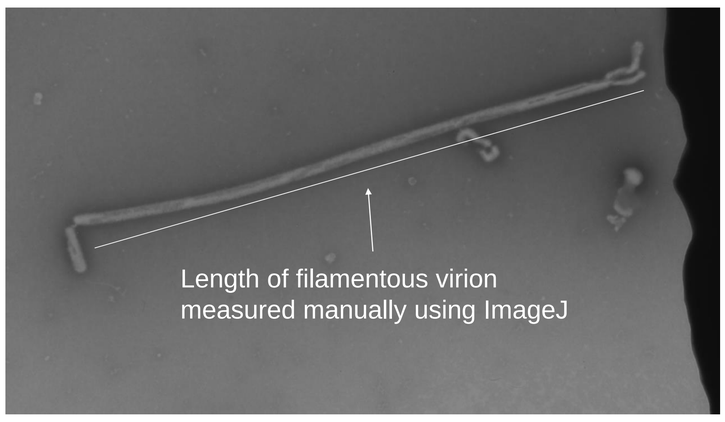


MDCK cells were infected with the filamentous influenza strain Udorn and the growth media harvested.

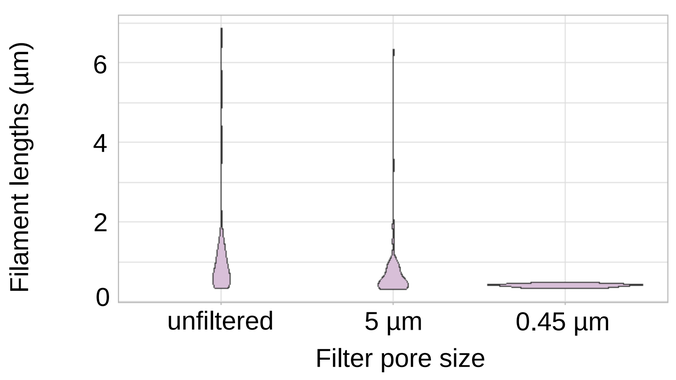
Three populations of influenza virions were created: unfiltered, 5 µm filtered, and 5 µm filtered followed by 0.22 µm filtered.

The 0.22 µm filter was later changed to a 0.45 µm filter due to the smaller filter becoming blocked. Dilution with PBS also improved filtration.

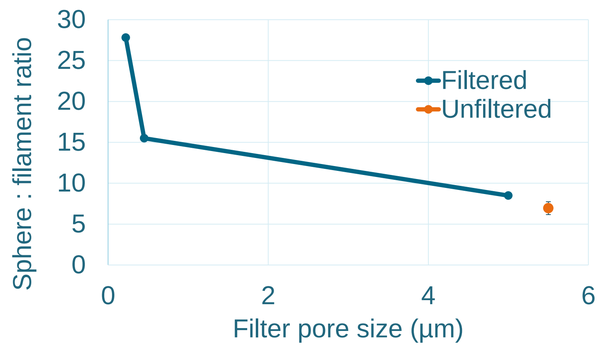
Negative Stain Transmission Electron Microscopy



3. Filtration depletes filamentous virions

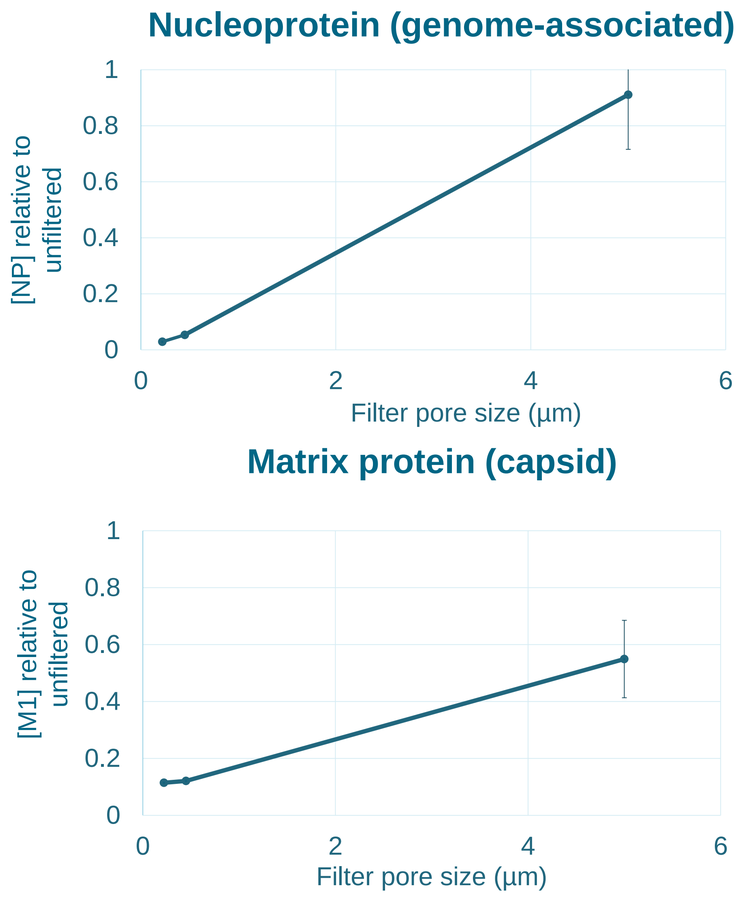


Filament lengths were measured using ImageJ. A 5 µm filter decreased the number of longer filaments observed and an 0.45 µm filter removed all long (> 500 nm) filaments.



- Filtration through small-diameter pores preferentially removes long filaments.

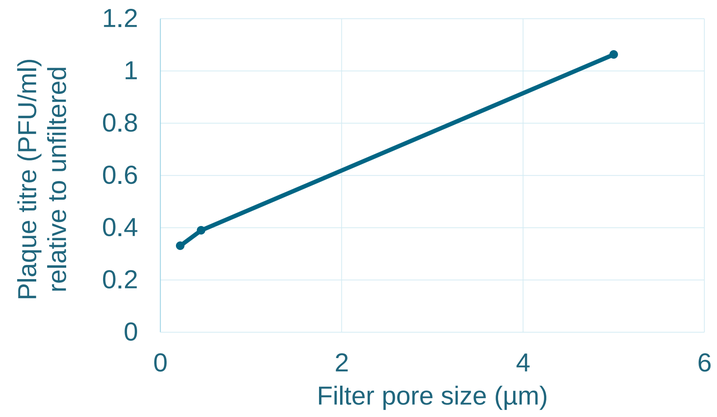
4. Filtration depletes viral protein



Viral nucleoprotein (NP; genome-associated) and matrix (M1; capsid) in filtrates were detected and quantified by Western blot.

- Concentration of both NP and M1 decreased substantially with decreasing filter pore size.
- This suggests that although filtration removes filaments it may not yield enough material for some downstream analyses.

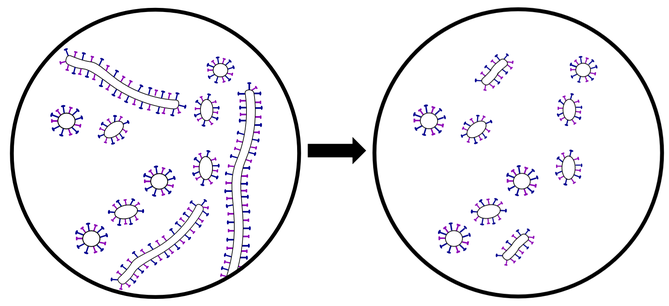
5. Filtration depletes infectious virions



- Infectivity also decreased substantially with decreasing filter pore size.

6. Conclusion

- Decreasing the filter pore size selectively removed long filamentous virions from the filtrate.
- This shows that it is possible to remove the filaments in influenza virus populations by filtration.



- Filtration substantially depleted the amount of viral protein and infectious virus in the filtrates, which could make it difficult to combine this method with all but the most sensitive downstream analyses of virion properties.
- Our preliminary results suggest that filaments may contribute to the overall protein composition and infectivity of an influenza virion populations, but more work is needed to test this.

Acknowledgements

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[1] BADHAM, M. D., ROSSMAN, J. S., 20116. *Filamentous Influenza Viruses.* Current Clinical Microbiology Reports, vol. 3, no. 3, pages 155-161.

[2] DADONAITE, B., VIJAYAKRISHNAN, S., FODOR, E., BHELLA, D., HUTCHINSON, E. C., 2016. *Filamentous influenza viruses.* Journal of General Virology, vol. 97, pages 1755-1764