Determining the role of mucin in the loss of infectivity of aerosolised coronavirus

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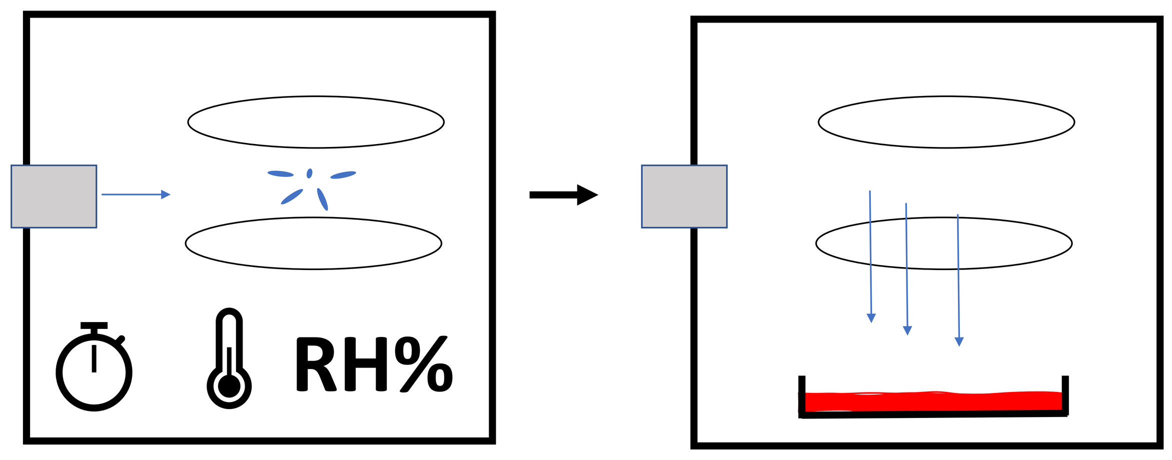
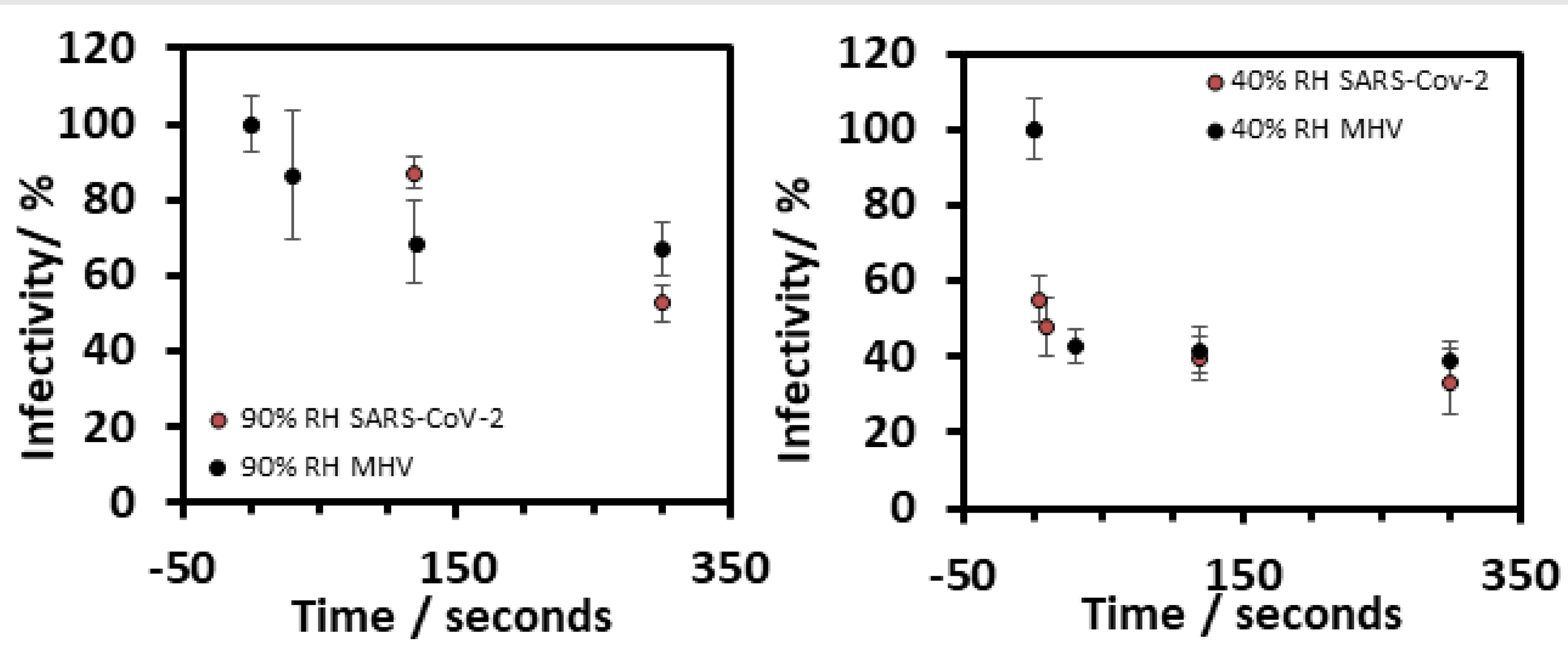
Background Respiratory infections are spread via the bioaerosols generated with coughing, talking, and breathing. By using a novel single particle levitation study system, we can interrogate the early lifespan of the bioaerosol with exceptional time resolution. By suspending bioaerosol in an electrodynamic field **Controlled Electrodynamic Levitation and Extraction of Bioaerosol onto Substrate (CELEBS)** allows for the highly reproducible measurement of viral viability under tight control of environmental conditions and over well-defined time periods. Equally, **Comparative Kinetics ElectroDynamic Balance (CKEDB)** uses similar technology to levitate single particles illuminated via laser, with the light scattering allowing for particle radius and phase to be inferred. This unique tandem approach for direct comparison between viral viability and evaporation kinetics of the respiratory aerosol as a function of time.

CELEBS



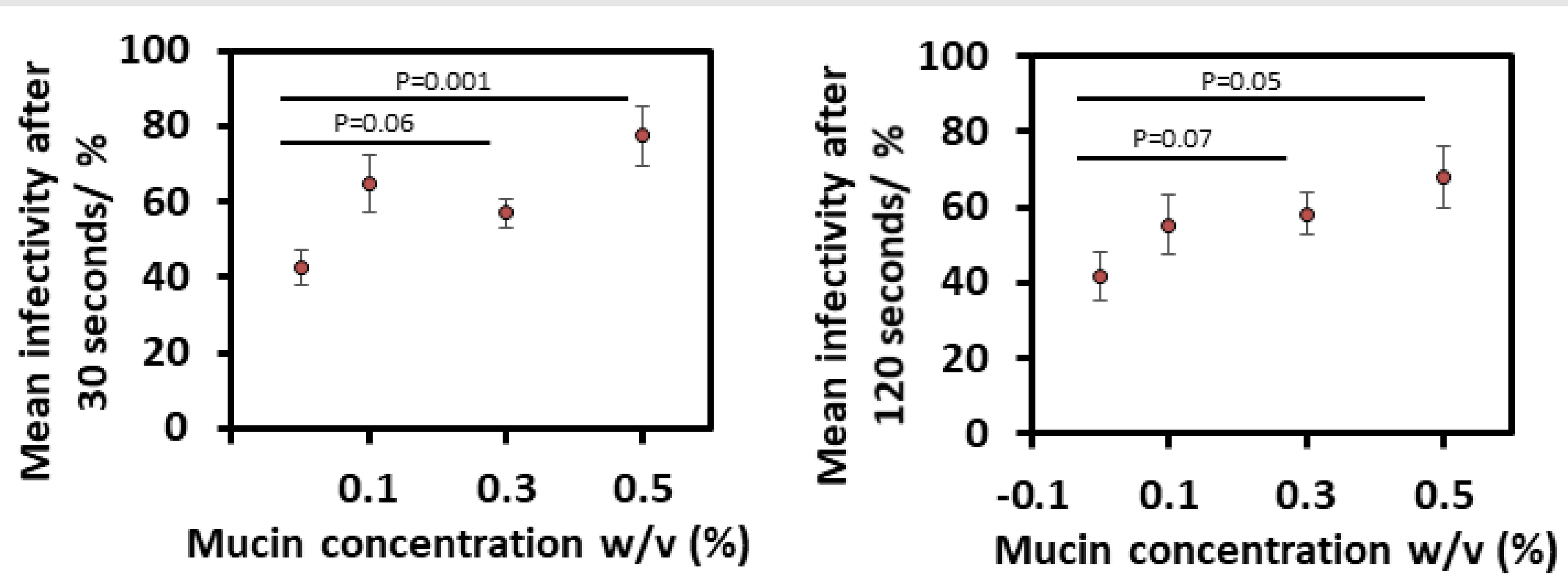
Bioaerosol of reproducible size are dispensed into the CELEBS chamber, where they are caught between two electrodes in an electrodynamic trap. Here they are subject to tight control of temperature and humidity, before deposition into cell tissue growth media ahead of downstream infectivity assay.

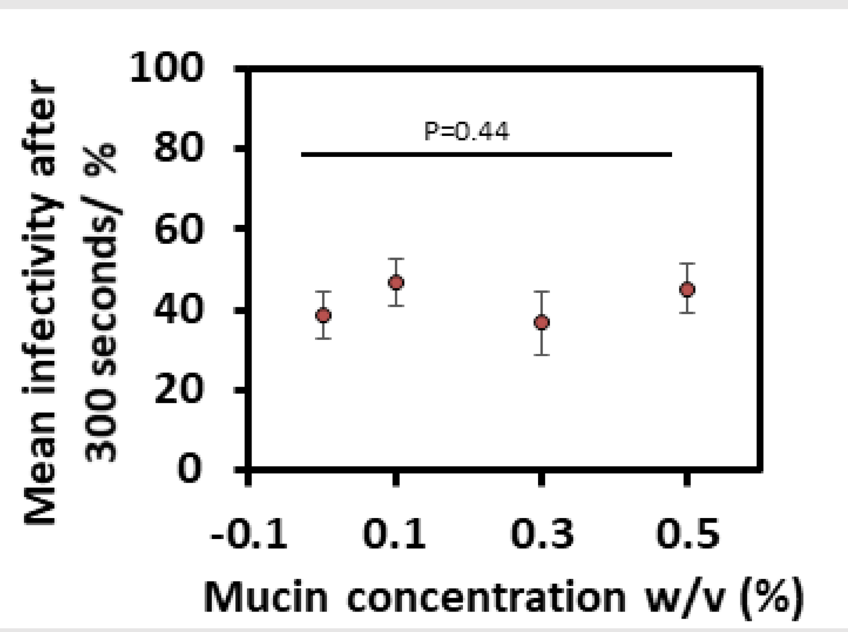
Mean infectivity with relative humidity



The rapid loss of infectivity at low RH (40%) points towards the role of efflorescence in airborne viral stability. A more gradual loss of infectivity at high RH (90%) could result from non-physiological conditions within the droplet such as increased particle pH as microchemistry equilibria imbalance.

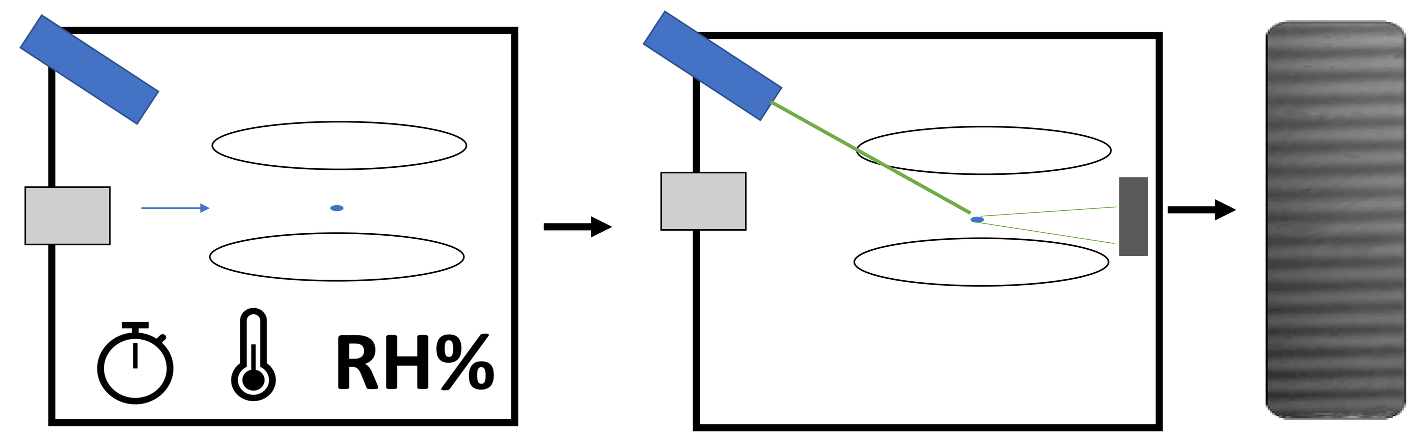
Mean infectivity with mucin concentration at 40% RH





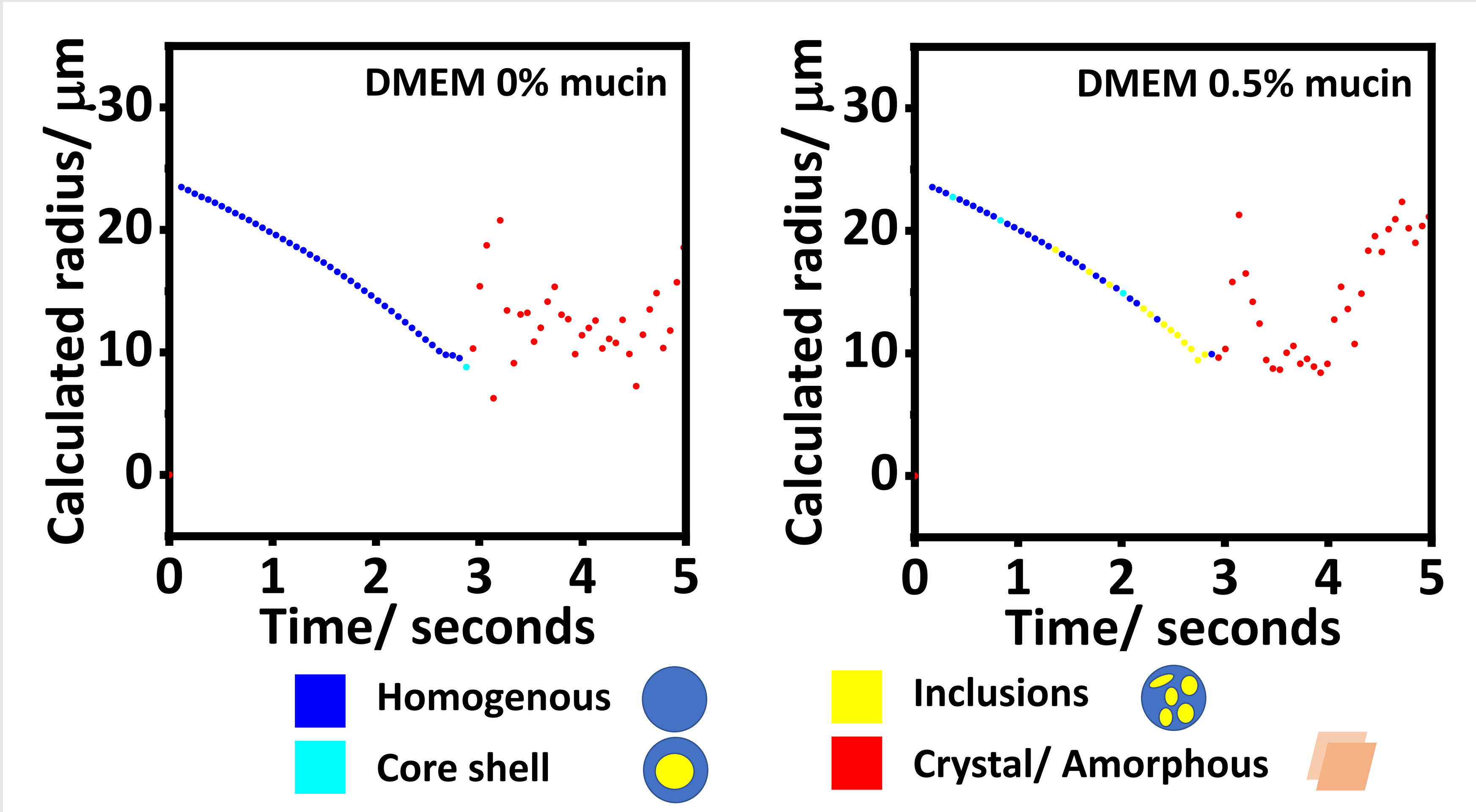
The addition of clinically relevant concentrations of mucin result in a reduced loss of MHV infectivity normally seen at 40% RH. This effect is seen transiently over two minutes with no observed change in infectivity after five minutes. Mucin is interacting with the virus to prevent inactivation as the droplet is changing phase.

CKEDB



Bioaerosol of reproducible size are dispensed into the CK-EDB chamber, where one droplet is caught between electrodes. As the droplet equilibrates, it is interrogated by 523nm laser, with the scattered light providing an evaporation profile for particle size and phase morphology determined from the Fourier transform.

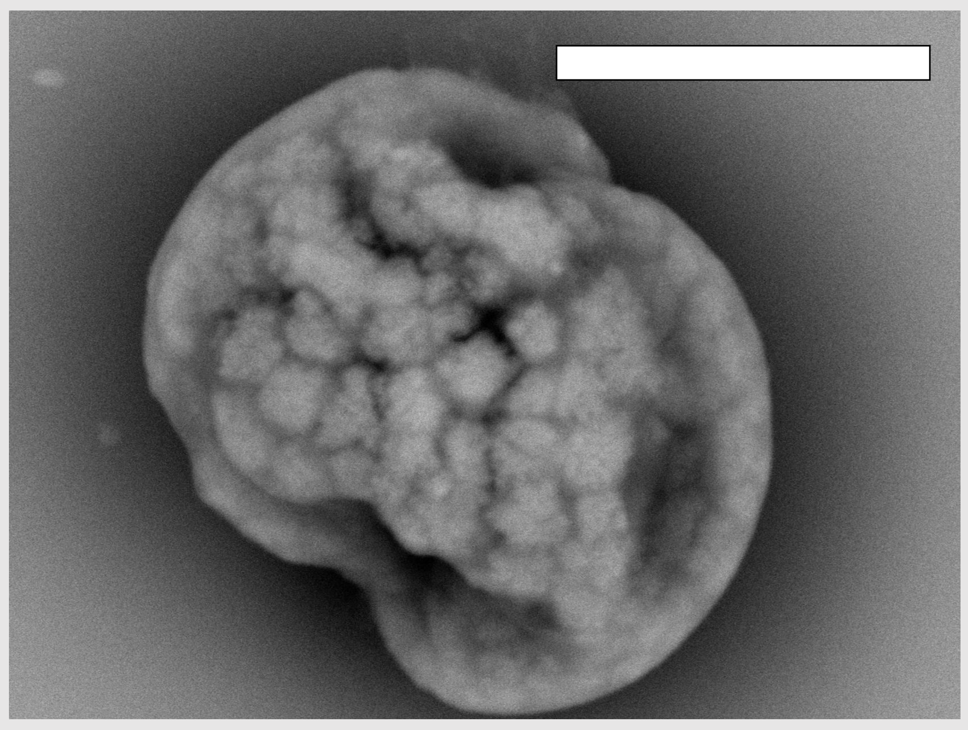
Evaporation profile for model respiratory droplets



The introduction of mucin results in an increased number of spontaneous inclusions within the droplet. It is these inclusion that offer the transient protection to the virus from disinfection within the aerosol particle.

Visual evidence of inclusions

SEM micrograph of a particle collected from the bottom of a falling droplet column after falling at 40% RH shows the phase separation within the particle (DMEM 10% FBS, 0.5% mucin) magnification x10,000, scale bar (white) indicates 5 µm.



Conclusion

- Increased mucin concentrations transiently reduced the loss of infectivity, driving the formation of inclusion bodies within the evaporating droplet.
- Confirmation of the role of RH, pH and phase change on viral infectivity.
- Established the influence of mucin on internal bioaerosol microphysics.
- As an ensemble these factors determine the viability of coronavirus in the aerosol phase

Otero-Fernandez,M.; Thomas, R.J.; Oswin, H.; Haddrell, A.E.; Reid, J.P. Transformative Approach to Investigate the Microphysical Factors Influencing Airborne Transmission of Pathogens. *Appl. Environ. Microbiol.* **2020**,
Oswin, H.P.; Haddrell, A.E.; Otero-Fernandez, M.; Mann, J.F.S.; Cogan, T.A.; Hilditch, T.; Tian, J.; Hardy, D.; Hill, D.J.; Finn, A.; et al. The Dynamics of SARS-CoV-2 Infectivity with Changes in Aerosol Microenvironment; 2022; doi:10.1101/2022.01.08.22268944