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viRepel AC / Dr Cu - Lab Test Report

Project Summary

viRepel has contracted the ImPaKT Centre to conduct viral titer reduction studies with SARS-CoV-2 after exposure to viRepel AC, also known by the name Dr Cu, material to determine if the surface coating demonstrates antiviral activity. From the results reported below, exposure to viRepel AC / Dr Cu for 1 hour results in a 90% reduction in infectious virus. Continued exposure to viRepel AC / Dr Cu for 6 hours results in a 99.9999% reduction of infectious virus depending on testing conditions.

Experimental Method

viRepel AC / Dr Cu material preparation

The viRepel AC / Dr Cu material was provided to ImPaKT as ~8x11in sheets with and without an adhesive backing. The viRepel AC / Dr Cu material is composed of a soft pliable rubber or plastic. For the viRepel AC / Dr Cu with an adhesive, the plastic side will be termed “top side” and the adhesive coated side will be termed “back side”. To prepare viRepel AC / Dr Cu for testing the top side was disinfected with 70% EtOH with a 5 min contact time inside a sterile biosafety cabinet (BSC). The material was cut into ~0.5x0.5cm squares and stuck to the sidewall (adhesive) or placed (non-adhesive) into 1.5ml tubes.

SARS-CoV-2 preparation and viRepel AC / Dr Cu Treatment

The SARS-CoV-2 virus stock at a titer of $10^{5.8}$ TCID50/ml was diluted to a multiplicity of infection (MOI) of 0.5 IU/cell. A volume of 850 ul of the diluted viral stock was added to a 1.5 ml tube containing the square of viRepel AC / Dr Cu adhered to the side wall of the tube. The tube containing the virus and viRepel AC / Dr Cu was placed on a tube rotator for set incubation times of 1min, 10min, 15min, 30min, 1hr, 3hr, 6hr, 12hr, and 24hrs. Untreated viral supernatant was used as a control. At each time point 100ul of supernatant was collected. Viral supernatants were diluted 100-fold to reduce any confounding cellular toxicity from prolonged exposure to viRepel AC / Dr Cu. Treated supernatants were titered onto 20,000 Vero E6 cells in 96 well flat bottom plates to quantitate viral titer reduction as a result of exposure to viRepel AC / Dr Cu.

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Results

In Table 1, after 1 hour of exposure to viRepel AC / Dr Cu viral titers were reduced by 1 Log corresponding to 90% reduction in infectious virus. With 6hrs of exposure to viRepel AC / Dr Cu the viral titer was reduced by >6 Logs, a 99.9999% reduction of infectious virus. Longer incubations with viRepel AC / Dr Cu did not produce any additional antiviral activity. Periods of incubation of less than 1 hour did not reduce infectious virus particles as determined by the methodology used in these experiments.

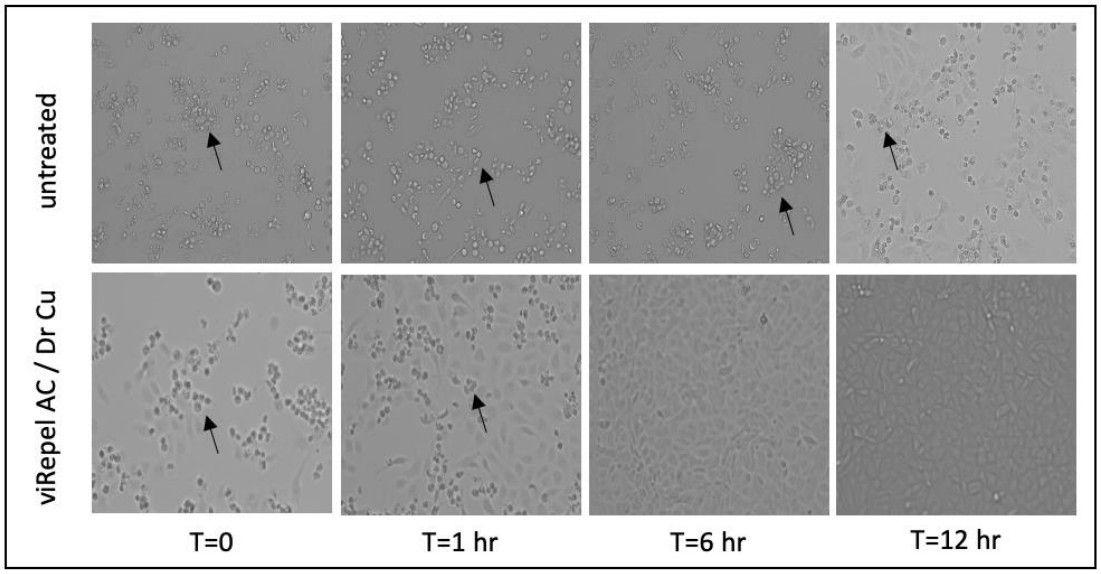
Table 1. viRepel AC / Dr Cu with Adhesive Viral Titer Reduction

Time	Viral Titer	Reduction Factor (Log10) v. Control	% Viral Reduction
1hr	$10^{1.8}$	1	90%
3hr	$10^{1.8}$	1	90%
6hr	0	>6	99.9999%
12hr	0	>6	99.9999%
24hr	0	>6	99.9999%

*Diluted control virus after dilution resulted in a TCID50/ml of $10^{2.8}$

Figure 1 displays representative fields of VERO E6 cells treated with SARS-CoV-2 virus that had been untreated or pre-incubated in tubes with viRepel AC / Dr Cu for designated time points. Viral cytopathic effects (vCPE), indicated with arrows, can clearly be seen in conditions when virus was untreated (i.e. not pretreated with viRepel AC / Dr Cu). A clear reduction in vCPE can be seen between untreated, 1hr +viRepel AC / Dr Cu, and 6hrs + viRepel AC / Dr Cu.

Figure 1. Representative fields of VERO E6 cells



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Supplementary material

List of study materials:

- viRepel AC / Dr Cu surface coating (1, 0.5 x 0.5cm square)
- SARS-CoV-2 (TCID50/ml $10^{5.8}$)
- VERO E6 cell line (*Cercopithecus aethiops*, kidney)
- Dulbecco's Modified Eagle media supplemented with 10% or 2% fetal bovine serum
- Polypropylene 1.5ml tubes
- 96-well flat bottom plates
- Tube rotator
- EVOS M7000 Microscope

Additional photos of experimental setup:

Figure S1. viRepel AC / Dr Cu material at receipt

Figure S2. viRepel AC / Dr Cu on side of tube

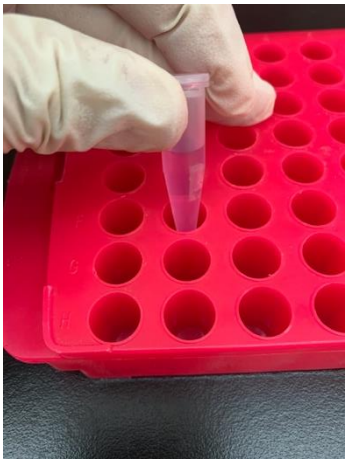

Figure S3. viRepel AC / Dr Cu in virus supernatant

Figure S4. viRepel AC / Dr Cu + virus rotating

Figure S4. Lab technician conducting experiments in lab