

Milan, July 9, 2020

Activity Report

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS-COV-2) QUANTIFICATION AFTER TUNGSTEN TRIOXIDE BASED (WO₃) PHOTOCATALYST TREATMENT

Test Facility

Viral Pathogenesis and Biosecurity Unit

San Raffaele Hospital, Milan

Study Director

Elisa Vicenzi, PhD

The aim of this study was to evaluate whether a WO_3 based photocatalyst system inhibits SARS-CoV-2 infectivity. The photocatalyst was placed on a metallic mesh filter to allow testing in liquid phase. Moreover, a cotton fabric soaked in a metallic nanocluster based (CuNh) copper solution (colloidal suspension) was added to improve SARS-CoV-2 inactivation.

Three devices were provided to the Viral Pathogenesis and Biosecurity Laboratory at the San Raffaele Hospital from NANOHUB. The SARS-CoV-2 isolate - obtained from the isolation of pharyngeal swab of a COVID-19 patient in Vero cells¹ – was inoculated in each of the specific devices specifically designed from the commissioner.

The viral stock was diluted 1:100 to obtain 80ml of virus suspension with a theoretical infectivity titer of 2.2×10^5 plaque forming unit (PFU) /ml. The viral suspension was introduced into the device from the top of the device and aliquots were collected from the bottom after 10 minutes up to 1 hour. The viral suspension was tested for the presence of infectious viral titers as tested in a plaque assay on Vero cells and quantification of viral RNA by real time PCR.

Vero cells were plated at 2.5×10^5 cells in each well in 24-well plates, in presence of EMEM culture medium with 10% (v/v) foetal serum (complete medium). Twenty-four hours later, cells were infected with the virus collected from the inactivation device at the several time periods. Serial dilutions (1:10 from non-diluted to 10^{-5}) of the virus collected after 10,15,20,30,60 minutes were tested in duplicate.

After an incubation period of 1 hour at $37^\circ C$, the supernatants were eliminated and 500 μ l of methyl cellulose at 1% (p/v) were added to each well in complete medium. After three days, cells were fixed with formaldehyde at 6% (v/v), saline solution, buffered with phosphate and stained with 1% (p/v) violet crystal in 70% (v/v) methanol.

The plaques were counted with a stereoscopic microscope (SMZ-1500, Nikon).

The calculation of the viral title expressed in plaque forming unit (PFU)/ml was determined by counting the plaques of those wells having a number lower than 100 plaques and multiplying that value for the corresponding dilution factor.

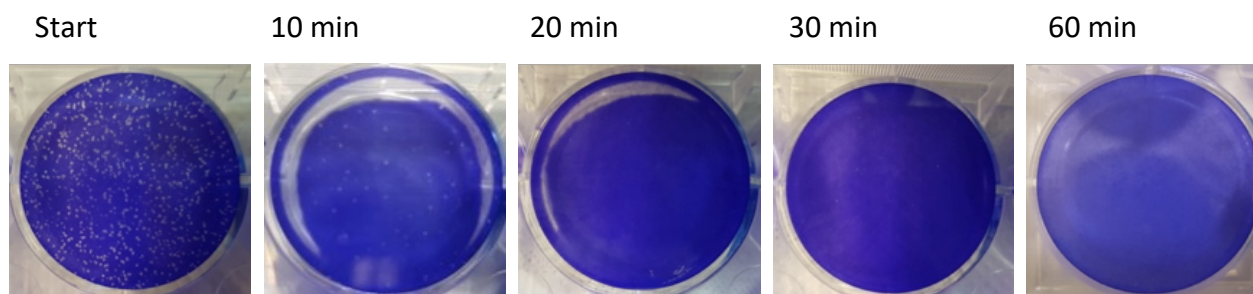
The collected material was tested for:

1. Inactivation of infectious viral titers by plaque assay in Vero cells
2. SARS-CoV-2 genome quantification by real-time PCR

1. Results of viral infectivity expressed in PFU/ml are summarized in the following table:

| Time period (minutes) | Experiment 1 | Experiment 2 | Experiment 3 |
|-----------------------|--------------|--------------|--------------|
| 0 | 12.200 | 13.766 | 11.683 |
| 10 | ND | 283 | 30 |
| 15 | 2000 | ND | ND |
| 20 | ND | 30 | 30 |
| 30 | 0 | 0 | 0 |
| 60 | 0 | 0 | 0 |

Results are the average of the values obtained in the plaque essay.



Plaque formation at start and following time as described above.

2. SARS-Cov-2 genome quantification through real-time PCR.

Viral RNA was extracted from the material collected at the different time points. Real-time PCR was next performed to determine the viral RNA copies present after the inactivation. The RNA quantification was carried out with the Quanty COVID-19 Kit from Clonit (Milan); the kit includes a standard reference curve of viral RNA at a known copy number. The SARS-CoV-2 target gene was the nucleocapsid (N).

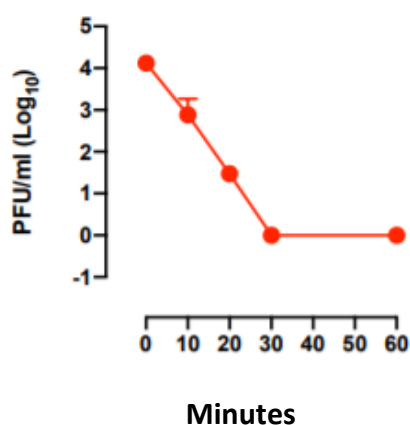
| Time period (minutes) | Experiment 1 | Experiment 2 | Experiment 3 |
|-----------------------|--------------|--------------|--------------|
| 0 | 7.253.850 | 8.718.142 | 10.909.490 |
| 10 | ND | 3.337.014 | 5.400.451 |
| 15 | 1.641.266 | ND | ND |
| 20 | ND | 1.972.495 | 4.433.552 |
| 30 | 1.910.394 | 1.097.204 | 2.783.446 |
| 60 | 165.987 | 243.029 | 669.995 |

Results are expressed as number of copies of viral RNA/ml.

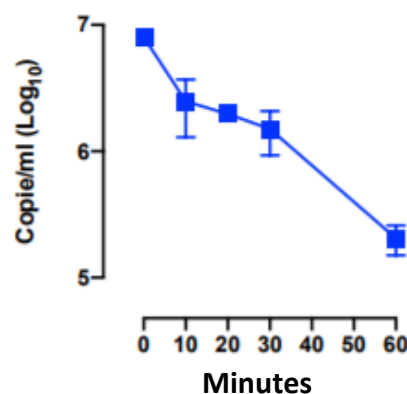
Summary Figure

The panel on the left shows the kinetic of the viral infectivity inactivation as measured in the plaque assay (data expressed in PFU/ml); the panel on the right shows the kinetic of viral RNA inactivation as measured by real-time quantitative PCR.

Infectious virus



Viral RNA



Summary and concluding remarks

NANOHUB device rapidly inactivates SARS-CoV-2 infectious load. Ten minutes after treatment, a reduction of 98.2% of infectious titers was observed, to reach 100% inactivation after only 30 minutes.

Furthermore, we tested the viral RNA amount present in the inoculum through real-time PCR. The side-by-side quantification of viral RNA load and infectious titers showed a ratio 1000:1; namely 1000 viral RNA molecule are needed to obtain 1 infectious particle. These data confirm and extend our previous observation on SARS-CoV^{2,3} suggesting a huge load of defective virions over infectious ones. However, NANOHUB device is capable of lowering the RNA load of approximately 1.5 log₁₀, suggesting that our photocatalysis system affects the virion's integrity even in its genomic component, although less efficiently as compared to the infectivity.

References

1. Mycroft-West CJ, Su D, Pagani I, et al. Heparin inhibits cellular invasion by SARS-CoV-2: structural dependence of the interaction of the surface protein (spike) S1 receptor binding domain with heparin. *bioRxiv* 2020.
2. Vicenzi E, Canducci F, Pinna D, et al. Coronaviridae and SARS-associated coronavirus strain HSR1. *Emerg Infect Dis* 2004; **10**(3): 413-8.
3. Pacciarini F, Ghezzi S, Canducci F, et al. Persistent replication of severe acute respiratory syndrome coronavirus in human tubular kidney cells selects for adaptive mutations in the membrane protein. *J Virol* 2008; **82**(11): 5137-44.

Dr. Elisa Vicenzi



Head of the Viral Pathogenesis and Biosafety Unit

Ospedale San Raffaele S.r.l.
Istituto di Ricovero e Cura a Carattere Scientifico

Via Olgettina 60 – 20132 Milano (MI) | Tel. +39 02.26431 | info@hsr.it
C.F., P.IVA e Reg. Imp. Milano 07636600962 – C.C.I.A.A. 1972938
Capitale Sociale € 60.817.200 i.v.

www.hsr.it

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Università Vita-Salute
San Raffaele