I.R.C.C.S. Ospedale San Raffaele

Gruppo San Donato

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Activity Report

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS-COV-2) QUANTIFICATION AFTER TUNGSTEN TRIOXIDE BASED (WO_3) PHOTOCATALYST TREATMENT

Test Facility

Viral Pathogenesis and Biosecurity Unit

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Study Director

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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 cells[1] – 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 $2.2x10^5$ 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 $2.5x10^5$ 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°C, the supernatants were eliminated and 500 µl 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:

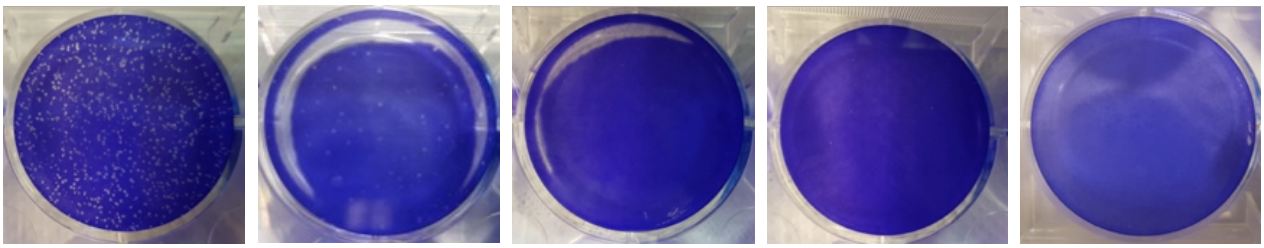
1. Inactivation of infectious viral titers by plaque assay in Vero cells
2. SARS-CoV-2 genome quantification by real-time PCR

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.

Start 10 min 20 min 30 min 60 min



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).

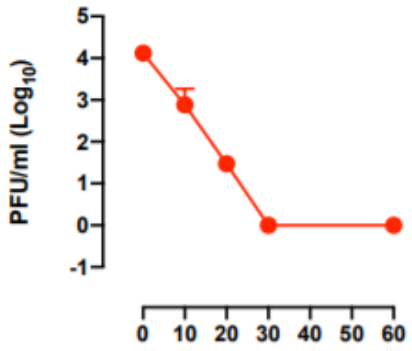
Time period (minutes)		Experiment 1		Experiment 2		Experiment 3
0		7.253.850		8.718.142		10.909.490
10		ND		3.337.014		5.400.451
15		1.641.266		ND		ND
20		ND		1.972.495		4.433.552
30		1.910.394		1.097.204		2.783.446
60		165.987		243.029		669.995

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

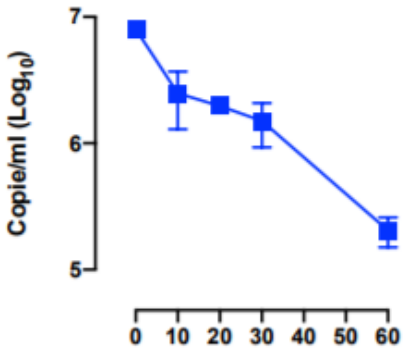
Summary Figure

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 inacrivation 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 $\log_{10}$, 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.

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