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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 TREATEMENT

Laboratory

Viral Pathogenesis and Biosecurity

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Aim of this study has been evaluating the employment of a WO_3 based photocatalyst for the inhibition of the SARS-CoV-2 virus. The photocatalyst was placed on a metallic mesh filter to allow test realization in liquid phase. Moreover, it was added a cotton fabric soaked in a metallic nanocluster based (CuNh) copper solution (colloidal suspension), to assess the separate effects. In certain air treatment applications, the cotton fabric soaked in copper solution is matched with a metallic filter on which a photocatalyst is placed, in order to increase the abatement effect of some microorganism and of viruses particularly.

Three devices were provided to the Viral Pathogenesis and Biosecurity Laboratory of San Raffaele Hospital from NANOHUB. The SARS-CoV-2 virus strain - 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 injection containing a theoretical infectivity titer of $2.2x10^5$ plaque forming unit (PFU) /ml. The viral suspension was introduced in the device and portions of it were collected in progressive time periods from 10 minutes to 1 hour. The gathered viral suspension was tested to verify the presence of infectious viral units through plaques test and viral RNA quantification.

The Vero cells were plated at $2.5x10^5$ cells in each well in 24-well plates, in presence of EMEM cultural ground integrated with 10% (v/v) foetal serum (complete ground). Twenty-four hours later, the 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} diluted) of the virus collected after 10,15,20,30,60 minutes from the system activation 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 ground. After three days, the 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 and multiplying that value for the corresponding dilution factor.

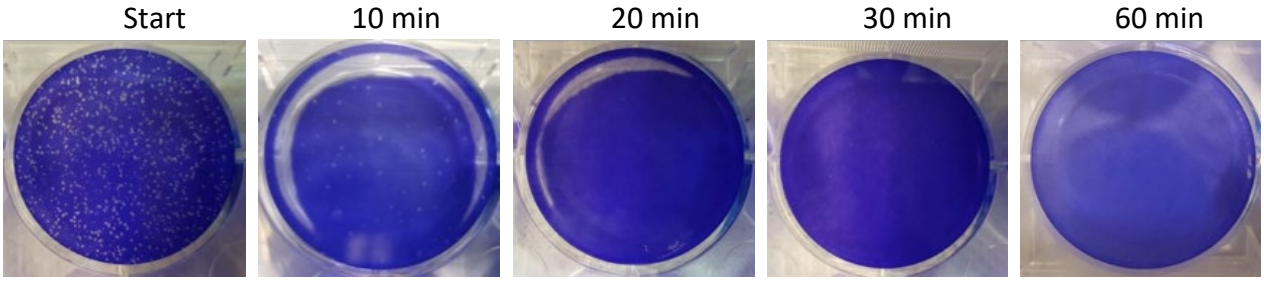
The collected material was tested for:

1. Abatement of the infectious viral load through plaques dosage in Vero cells
2. SARS-Cov-2 genome quantification through 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		30		30
20		ND		0		0
30		0		0		0
60		0		0		0

Results are the average of the values obtained from the viral injection dilution in the plaque essay multiplied for the dilution factor employed.



Picture representing the plaques' presence at the start and at the following time periods, as described above.

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

Material gathered at different time periods was submitted to viral RNA extraction, followed from real-time PCR to define the viral RNA amount present after the inactivation. The RNA quantification was executed with the Quanty COVID-19 Kit of Clonit (Milan); the kit comprehends a standard reference curve of viral RNA at a number of known copies. The gene amplified from the system is the nucleocapsid (N).

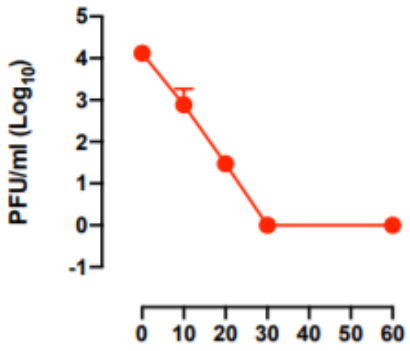
Time period (minutes)		Experiment 1		Experiment 2		Experiment 3
0		7.253.850		8.718.142		10.909.490
10		ND		3.337.014		5.400.451
15		1.641.266		ND		ND
20		ND		1.972.495		4.433.552
30		1.910.394		1.097.204		2.783.446
60		165.987		243.029		669.995

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

Summary picture

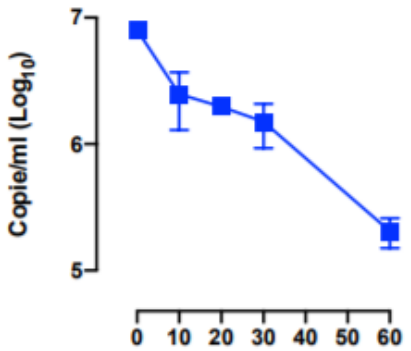
The panel on the left depicts the inactivation time course of the viral infectivity measured in the plaques dosage (data expressed in PFU/ml); whether the chart on the right shows the inactivation time course of viral RNA measured with real-time quantitative PCR.

Infectious virus



Minutes

Viral RNA



Minutes

Summary and concluding remarks

NANOHUB device is capable of inactivating the SARS-CoV-2 infectious load in quick times. At 10 minutes after treatment, one can observe an infectious load reduction of 98.2%, to get to a reduction of 100% at only 30 minutes.

Moreover, we tested the viral RNA amount present in the injection through real-time PCR. The viral RNA measure within the injection is higher compared to the infectious load in a ratio 1000:1; namely 1000 viral RNA molecule are needed to have 1 infectious particle. These data confirm what was previously observed for SARS-CoV[2,3] and are explained from the presence of weak virions which do contain RNA but are not infectious. However, NANOHUB device is capable of lowering the RNA amount of approximately 1.5 logarithms to base 10, suggesting that photocatalysis affects the virion's integrity even in its genomic component, although less efficiently 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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