Dr. Shashank Tripathi
Assistant Professor
Microbiology & Cell biology Department (MCB)
Centre for Infectious Disease Research (CIDR)
Indian Institute of Science (IISc)
CV Raman Avenue, Bangalore, Karnataka, 560012, India
Phone: 080-22932884; Email: shashankt@iisc.ac.in

PROJECT REPORT **10/07/22**

Client: Sharp Business Systems (India) Pvt. Ltd., New Delhi

Purpose of study: Testing Photocatalyst (Tungsten trioxide) coating on the glass for cytotoxicity and virucidal activity against SARS-CoV-2.

Summary: In this project virucidal efficacy of Tungsten trioxide coating on Glass was tested against the SARS-CoV-2 Omicron variant in the BSL3 facility at CIDR, IISc. Results indicated that infectious virus counts on the Tungsten trioxide coated plate were reduced to ~4.14% after 4 hours of contact, and to non-detectable levels after 8 hours of contact, in presence of ambient light. Cytotoxicity of the Tungsten trioxide coating on Glass was also tested on mammalian cells and no toxicity was observed of the media incubated with the coated plates up to the highest concentration of 1/10.

Principal Investigator:
Dr. Shashank Tripathi

Dr. Shashank Tripathi
Assistant Professor
Department of Microbiology & Cell Biology
Center for Infectious Disease Research
Indian Institute of Science
Bangalore - 560 012

SHARP BUSINESS SYSTEMS (INDIA) PROJECT

VIRUCIDAL ACTIVITY OF TUNGSTEN TRIOXIDE-COATED PLATES

Methods:

1. Virus

SARS-CoV-2, (Isolate hCoV-19/USA/MD-HP20874/2021, Lineage B.1.1.529; Omicron variant NR-56461, BEI Resources, NIAID, NIH) was propagated and titrated by plaque assay in Vero E6 cells.

2. Tungsten trioxide(*)-coated plate cytotoxicity assay

(*)Tungsten trioxide is a type of Photocatalyst.

A 100 µL of 1X DMEM medium was added at the center of a Tungsten trioxide-coated plate and incubated for 24h under LED light at ~1000 LUX in a biological safety hood. After incubation, the DMEM was recovered and serially diluted using ice-cold-DMEM and assessed for cytotoxic effect at their different dilutions (10^{-1} to 10^{-8}) by plating these samples on VERO-E6 cells coated 96-well plate (~80% confluency). After 48h of incubation, MTT reagent was added and absorbance was measured at 570nm. Cells with DMEM alone were used as a reference control.

3. Experimental setup

A day before the experiment, all the tungsten trioxide-coated and uncoated plates (prepared and provided by the SHARP CORPORATION) were sterilized by UV irradiation overnight. The next day, in a biosafety cabinet (BSL3 lab), a stack of filter papers was placed in sterile Petri dishes and made wet using sterile water to hold the moisture content till the incubation period. Two autoclaved metal rings were placed in the middle of each Petri dish over which tungsten trioxide-coated or uncoated plates was placed. A 100 µL of SARS-CoV-2 suspension ($6.6x10^6$ PFU) or DMEM was loaded at the center of the glass plate and then gently put a clean coverslip over it to avoid drying followed by placing an upper lid on the Petri dish. Two table lamps were placed on either side of the experimental setup and the light intensity was maintained in the range of 1000-1200 LUX for activation of tungsten trioxide (Photocatalyst) that was coated on the plates (Fig. 1).

Eight sets of samples in triplicates (24 Petri dishes) were tested. Among these, five sets of Petri dishes included the virus suspension+tungsten trioxide-coated

plate and were exposed to light for 0, 4, 8, 12 and 24 h. One set was for virus suspension+tungsten trioxide-coated plate but the Petri dish was wrapped completely with aluminum foil (to avoid photocatalytic activation of tungsten trioxide) and was incubated under the lamp for 24 h. For the other two sets, only DMEM was added to the tungsten trioxide-coated plate or Virus suspension over an uncoated plate and exposed to light for 24 h.

Figure 1: The figure showing the experimental setup made in the biosafety cabinet and light intensities measured on corners and middle of the setup.

4. Virucidal activity by plaque assay

The virucidal activity of tungsten trioxide-coated plates was measured by plaque assay twice in a BSL3 laboratory. Here, 12-well plates were seeded with $0.2x10^6$ VERO E6 cells/mL/well and incubated at 37ºC with 5% CO_2 for 48h to achieve 100 % confluency. A 100 µL of the test sample was serially diluted by 10 fold up to 10^{-6} in DMEM containing 2% FBS. Old media was removed followed by the addition of 100 µL of diluted sample to wells and incubated for 1h at 37ºC with intermittent shaking. Then, virus suspension was removed and added 1 mL of 0.6 % avicel in DMEM containing 2% FBS and incubated for 72h. After that, avicel was removed completely and fixed with 1mL of 4 % formaldehyde in PBS. After 30 min of incubation under UV light, cells were stained with 1% crystal violet, removed after 5 min and washed with tap water to count the number of plaques obtained and were represented as PFU/mL using the formula; the number of plaques per well / dilution tested x volume of diluted sample added.

5. Statistical analysis

The data were analysed using GraphPad Prism v 8.4.3 and represented as mean ± SD. Statistical variations were determined by one-way ANOVA with Tukey's multiple comparisons tests.

Results:

1. Cytotoxicity assay

The tungsten trioxide-coated plate didn't show any toxicity on VERO E6 cells at any of the dilutions used and hence it is non-toxic. Also, the morphology of VERO E6 cells, such as cell attachment and shape looked normal even after 48h of incubation.

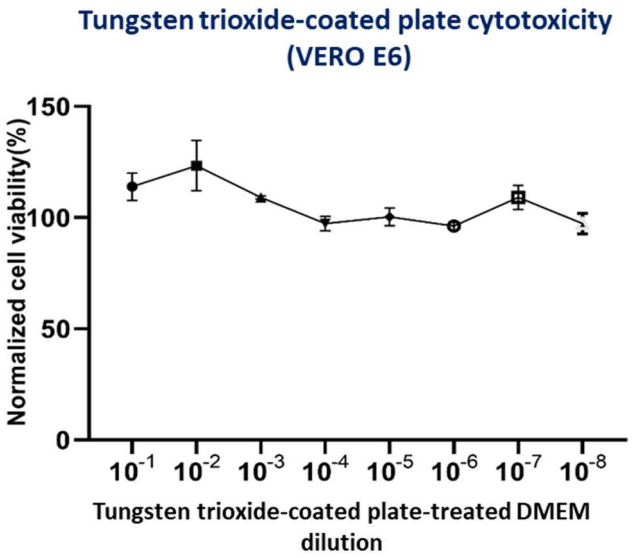

Figure 2: Cytotoxicity assay of tungsten trioxide-coated glass plates. A 100µL of DMEM medium was placed over a tungsten trioxide-coated plate kept inside the Petri dish setup, covered with a cover slip and exposed under ~1000 LUX LED light for 24h inside a biosafety hood. Later, the VERO-E6 cells in a 96-well plate were treated with different dilutions of the recovered DMEM medium and the cytotoxic effect was determined at 48h post-incubation by MTT assay.

2. Virucidal assay

The plaque assay data showed a reduction in Omicron virus titer by 95.86% when exposed to a tungsten trioxide-coated plate kept under ~1000 LUX LED illumination

for 4h. In the remaining time points of virus exposure to the tungsten trioxide-coated plates i.e, for 8, 12 and 24 h, the viral titer has reduced to lower than detection limit.

So, the maximum reduction in viral titer was observed when the Omicron virus was exposed to at least 8h over a tungsten trioxide-coated plate kept under LED illumination. However, the viral titer has also reduced to lower than detection limit when tungsten trioxide-coated plate containing Petri dishes were wrapped with aluminium foil. Further, the virus suspension placed over uncoated plates for 24h under illumination, also had a viral titer reduced by 71.02 % (Fig. 3 and Table 2).

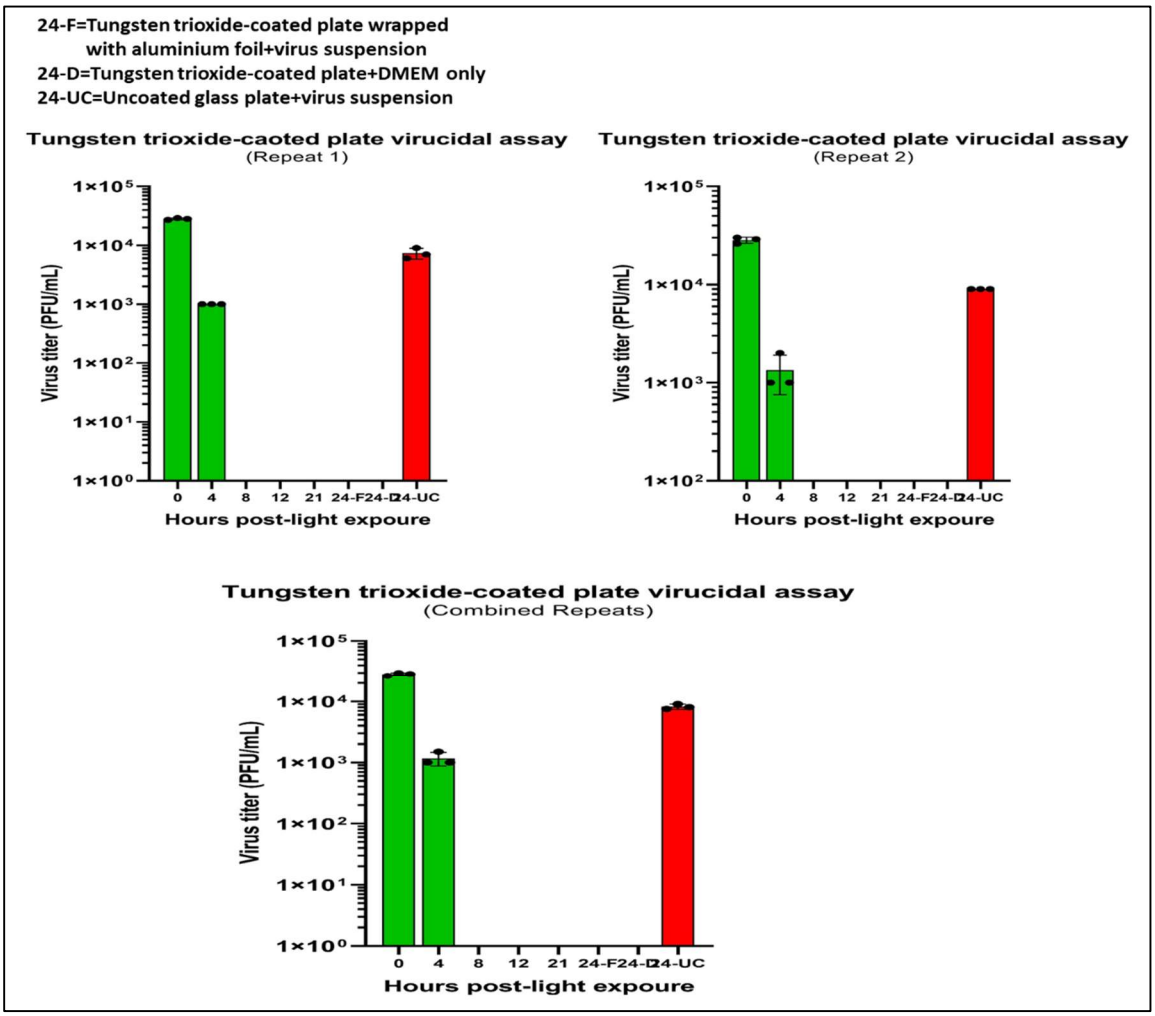

Figure 3: Virucidal activity of tungsten trioxide-coated glass plates by plaque assay. A 100 µL of SARS-CoV-2 virus suspension (Omicron strain=$6.6x10^6$ PFU/mL) was added over the tungsten trioxide-coated or uncoated glass plates in the petri dish set up and exposed to LED light (1000-1200 LUX) for 0, 4, 8,12 and 24 h after which they were serially diluted (10 fold) in DMEM. The tungsten trioxide-coated plates added with DMEM only were also used as a negative control. The diluted samples were used for plaque assay on VERO-E6 cells. After 48h of incubation, the number of plaques formed was counted and converted to PFU/mL.

Table 2: Percent reduction of SARS-CoV-2 (Omicron variant) virus as observed by plaque assay after placing them over tungsten trioxide-coated or uncoated plates under LED light (~1000-1200 LUX) at different time points (0,4,8,12 and 24 h). The data from experimental repeat-1, repeat-2 and their combined data are shown**.** **The percent reduction was calculated keeping "viral titer observed at 0h post-treatment" as a reference control.*

REPEAT 1

SL No.	Test organism with tungsten trioxide-coated plate exposure time	Average viral titer (PFU/mL)	*Percent reduction (%)
1	SARS-CoV-2 Omicron strain (0 hour)	$2.80x10^4$	-
2	SARS-CoV-2 Omicron strain (4 hour)	1.0×10^3	**96.43**
3	SARS-CoV-2 Omicron strain (8 hour)	0	**ND**
4	SARS-CoV-2 Omicron strain (12 hour)	0	**ND**
5	SARS-CoV-2 Omicron strain (24 hour)	0	**ND**
6	SARS-CoV-2 Omicron strain (24 hour-Foil wrapped)	0	**ND**
7	SARS-CoV-2 Omicron strain (24 hour-Uncoated plate)	$7.30x10^3$	**73.81**

REPEAT 2

SL No.	Test organism with tungsten trioxide-coated plate exposure time	Average viral titer (PFU/mL)	*Percent reduction (%)
1	SARS-CoV-2 Omicron strain (0 hour)	$2.83x10^4$	-
2	SARS-CoV-2 Omicron strain (4 hour)	1.33×10^3	**95.29**
3	SARS-CoV-2 Omicron strain (8 hour)	0	**ND**
4	SARS-CoV-2 Omicron strain (12 hour)	0	**ND**
5	SARS-CoV-2 Omicron strain (24 hour)	0	**ND**
6	SARS-CoV-2 Omicron strain (24 hour-Foil wrapped)	0	**ND**
7	SARS-CoV-2 Omicron strain (24 hour-Uncoated plate)	$9.00x10^3$	**68.23**

Combined repeats

SL No.	Test organism with tungsten trioxide-coated plate exposure time	Average viral titer (PFU/mL)	*Percent reduction (%)
1	SARS-CoV-2 Omicron strain (0 hour)	$2.815x10^4$	-
2	SARS-CoV-2 Omicron strain (4 hour)	$1.16x\ 10^3$	**95.86**
3	SARS-CoV-2 Omicron strain (8 hour)	0	**ND**
4	SARS-CoV-2 Omicron strain (12 hour)	0	**ND**
5	SARS-CoV-2 Omicron strain (24 hour)	0	**ND**
6	SARS-CoV-2 Omicron strain (24 hour-Foil wrapped)	0	**ND**
7	SARS-CoV-2 Omicron strain (24 hour-Uncoated plate)	$8.15x10^3$	**71.02**

***ND= Not Detected**