REPORT:

SARS-CoV-2 inactivation on aluminum surfaces by IonO2 SeLF device

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ABSTRACT

Designing effective methods of SARS-CoV-2 inactivation that can be applied in daily human activities can help diminish the transfer and spread of infectious diseases such as COVID-19. ReSPR technology has shown to be effective in reducing pathogens and allergens from the air and from surfaces. It is used in devices that release oxidizing particles to purify the air that people inhale. We tested the SARS-CoV-2 inactivation efficacy of a IonO2-Self device located at 18 cm, 24cm and 36 cm from an aluminum surface coated with 10^5 PFUs of SARS-CoV-2 during different exposure times. A plaque assay was used to measure viral titers after 240 and 360 minutes of exposure with and without the presence of the device.

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MATERIALS AND METHODS

Material infection and sample collection

IonO2 self device was placed inside a Biosafety cabinet (BSC) and turned on. Sterile aluminum foil pieces of 24mm x 24mm previously disinfected with 70% ethanol and exposed to UV light for 25 minutes, were individually placed in a petri dish inside the BSC and were kept at room temperature. A 50µl inoculum of 1 x 10^5 PFU of SARS-CoV-2 was placed and extended on each aluminum piece using a micropipette tip. Sets of four replicates were prepared and located 18, 24 and 36 cm from the IonO2-self device.

Enough samples were prepared to evaluate three exposure times (0, 240 and 360 minutes) for each distance (Table 1); another set of four replicates were prepared without the presence of the IonO2 device for the 0, 240 and 360 minutes of exposure test to serve as a control. Following each exposure time, 3ml of collection media (DMEM with 2%FBS) was added to each petri dish, making a dilution of 1:60, and the aluminum material was washed out by resuspending four to five times using a micropipette; the viral suspension was collected, mixed for homogeneity and aliquoted into 1ml centrifuge tubes. Each collected sample was immediately labeled and stored at -80°C for titration assays.

Table 1. Evaluated treatments

Virus dose	Exposure time (min)	Treatment
$1x10^5$ PFU/50µl	0, 240, 360	IonO2 self
		No Device (Control)

Viral-inactivation quantification

The recovered virus suspension was diluted (10-fold, 3 dilutions: 1/60, 1/600, 1/6000) in a mixing plate in duplicate and added to 96 well Vero E6 seeded plates. Plates were incubated for 1 hour at 37°C. Inoculum was discarded and a 2% carboxymethylcellulose overlay was added and incubated for 24 hours at 37°C. Next, the overlay was discarded, plates washed and fixed for 10 minutes at -20°C (using acetone-Methanol solution). Following fixation, plates were washed two times with PBS-T and a primary antibody (IgG Human anti-Coronavirus, 1:2500) was added and incubated overnight at 37°C. The primary antibody was then discarded, and plates were washed twice with PBS-T. A secondary antibody (Goat IgG Anti-Human HRP conjugated, 1:2000) was added and left to incubate for 2 hours at 37°C. After removing the secondary antibody, plates were washed twice with PBS-T and plaques were developed with a Chromogen substrate. Plaques were counted using Immunospot Image analyzer and open-source software Viridot to determine the viral titer. The titer reduction percentage was calculated using the following formula:

$$Percent\ reduction = \frac{(A - B)\ x\ 100}{A}$$

Where: A is the mean virus titer with no treatment (Control) or the initial titer; and B is the viral titer after treatment.

Data analysis

A Two-way ANOVA and a Tukey multiple comparison test were performed to compare the IonO2 device with the control.

RESULTS

Data of all evaluated exposure times showed a faster deactivation of the SARS-CoV-2 inoculum when exposed to the IonO2 Self device

Time	IonO2 Self at 18cm		IonO2 Self at 24cm		IonO2 Self at 36cm	
	Mean	SD	Mean	SD	Mean	SD
240	45.22%	17.34	39.13%	17.82	54.78%	12.38
360	74.35%	4.98	49.57%	7.71	58.26%	8.52

Table 1. Mean titer reduction percentage and standard deviation of SARS-CoV-2 inoculum collected after 240 and 360 minutes of exposure from 24mm x 24mm aluminum foil pieces exposed to a IonO2 self-device.

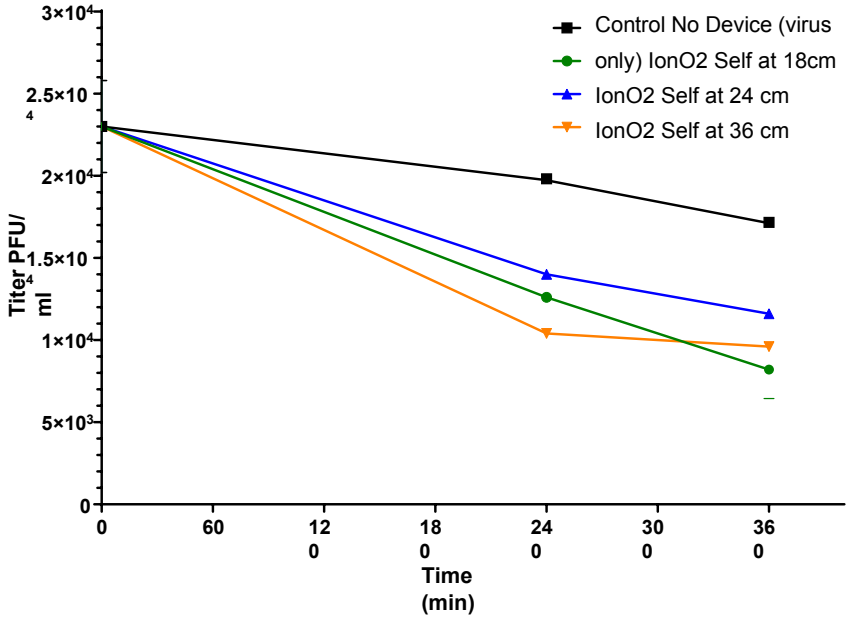

Figure 1. Mean titers and standard deviation of SARS-CoV-2 inoculum collected after 240 and 360 minutes of exposure from 24mm x 24mm aluminum foil pieces exposed to a IonO2 Self device and comparison with control.

CONCLUSION

The IonO2 self-device showed an effective SARS-CoV-2 inactivation activity during the evaluated periods when placing the device at 18, 24 and 36 cm from the infected materials.