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Assessment of Melt Blown Fiber with Antiviral Coating Against SARS-CoV-2

Letter Report

FOR

Aurabeat Technology Limited

Roger Sze To
651, 6/F., Building 19
Science Park W Ave.
Pak Shek Kok, Hong Kong

MRIGlobal Project No. 311664.01.001

September 4, 2020

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Roger Sze To
651, 6/F., Building 19
Science Park W Ave
Pak Shek Kok, Hong Kong

Subject: MRIGlobal Project No.: 311664.01.001 Final Report

Dear Mr. Sze To:

MRIGlobal is pleased to submit this letter report to Aurabeat Technology Limited titled "Assessment of Melt Blown Fiber with Antiviral Coating against SARS-CoV-2." The objective of this project was to determine if Filter Media treated with Aurabeat AG+™ Antiviral Air Filtration technology has the ability to inhibit the growth of SARS-CoV-2 *in vitro*. We added 200 µl of virus stock to melt blown fiber coupons with coating X or uncoated as control. After 30 minutes, we recovered virus (if any) from the coupons and added it to Vero E6 cells. Plates were examined at 5 days post-testing for cytopathic effects (CPE). Cytotoxicity was seen with X treatment but not in control samples. No CPE was observed with samples from the X treated samples past cytotoxicity at 30 minutes.

1. Results Summary

The objective for project 311664.01.001 was to determine if Filter Media treated with Aurabeat AG+™ Antiviral Air Filtration technology has the ability to inhibit the growth of SARS-CoV-2 *in vitro*. MRIGlobal utilized the USA-WA1/2020 strain of the virus, acquired from BEI Resources (NR-52281). This was propagated in Vero E6 cells (ATCC CRL-1586); these cells were also used for the neutralization assay. Vero E6 cells were cultured in growth media consisting of Dulbeco's Modified Eagle Medium/F12 supplemented with 5% FBS (Fetal Bovine Serum), and PSN (penicillin, streptomycin, and neomycin).

The Vero E6 cells were plated on 96-well plates the day before the assay and were allowed to grow to ~ 60%-70% confluence. On the day of the assay, melt blown fiber coupons were inoculated with 200 µl of virus stock and allowed to sit for 30 minutes in a biosafety cabinet. When 30 minutes passed, 2 ml of DMEM/F12 media was added to coupons and lightly scraped with a cell scraper to aid in viral resuspension. Samples were added to an empty 96-well plate and diluted 1:10 down the plate in DMEM/F12. These dilutions were then transferred to a plate of Vero cells with media removed. After at least 15 minutes, DMEM/F12 supplemented with FBS was added to cells to feed them for the next 5 days. This incubation period of at least 15 minutes is to allow the virus to adsorb to cells without interference from FBS. Cytotoxicity

controls of the test articles without virus added were also performed. The assay was executed in five replicates for each condition.

After 5 days, cells were examined for the presence of cytopathic effect (CPE) associated with viral presence and replication. Examination is done using a microscope (10x objective to view the entire well at once) and observing the morphology of the cells. Healthy Vero cells have somewhat transparent appearance with pinched or rounded ends in a monolayer of cells with little to no space between cells. Dead cells displaying CPE are often not adhered to the plate, round, and much smaller than living cells. Considerable empty space can be seen on the bottom of the plate where cells have detached from the surface. Any well displaying CPE is marked as positive whether the whole well, or only a small portion is affected, because this is indicative of the presence of viable virus.

Cytotoxicity was observed at the first dilution of recovered samples from X treated coupons. When media was added to the X coupons, it changed from a red color to a purple color, which is indicative of an increase in pH to a more basic level. No cytotoxicity was observed from control samples. Since there was no CPE observed after the cytotoxic effects were gone, we cannot say for certain whether there was virus present in the wells that displayed cytotoxicity, thus limiting the quantification of viral reduction in these samples. Day 5 reads showed no CPE in any test wells. All uninfected controls remained healthy and did not display any CPE throughout the 5-day observation period. Table 1 summarizes these findings. Results were calculated using the Reed & Muench Calculator (produced by BD Lindenbach from "Measuring HCV infectivity produced in cell culture and in vivo" Methods Mol Biol. (2009) 510:329-36). Results are shown as Log reduction relative to timed controls as well as a percent reduction of SARS-CoV-2 infectivity.

Table 1. Results of *in vitro* Neutralization of SARS-CoV-2 with coated melt blown fiber.

Sample Name	Time of Contact (minutes)	TCID50	Log10 TCID50	Average TCID50	Average Log10 TCID50	Log Reduction to Virus Controls	Percent Log Reduction
X30-1	30	31.62	1.50	31.62	1.50	3.33	99.95%
X30-2	30	31.62	1.50				
X30-3	30	31.62	1.50				
C30-1	30	50118.72	4.70	75376.66	4.83		
C30-2	30	125892.54	5.10				
C30-3	30	50118.72	4.70				

Based on this experiment, we conclude that melt blown fiber coated with X treatment is very effective at inhibiting SARS-CoV-2 infection of Vero cells. It is important to note that the cytotoxicity observed from X treatment limits the enumeration of virus at the highest concentration and therefore it cannot be said whether virus was present in those wells which showed cytotoxicity.

Sincerely,

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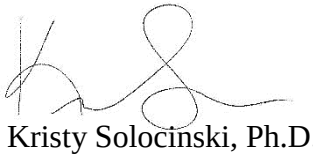

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