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Brief Report

Efficacy of an antimicrobial surface coating against human coronavirus 229E and SARS-CoV-2



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A B S T R A C T

The COVID-19 pandemic has accelerated the demand for alternatives to standard cleaning and disinfection practices. Antiviral coatings may provide an alternative to common surface treatments. A newly developed quaternary ammonium polymer coating was applied to stainless steel coupons and evaluated for efficacy against human coronavirus 229E and SARS-CoV-2. The polymer coating reduced levels of both test viruses by greater than 99.9% relative to non-coated stainless steel coupons during a 2-hour contact time.

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BACKGROUND

Respiratory viruses remain viable on inanimate, environmental surfaces (ie, fomites) for extended periods of time depending on temperature, humidity, and other factors.¹ Effective cleaning and surface disinfection practices reduce levels of infectious viruses and the potential for their spread.² Nevertheless, recontamination can occur at any time following the use of liquid disinfectants, which are typically wiped dry from surfaces after application. A number of self-disinfecting surface formulations have been developed during recent years.³ A continuously-active quaternary ammonium polymer applied to high-touch surfaces by certified technicians using electrostatic sprayers previously resulted in the reduced spread of antibiotic-resistant bacterial infections in clinical settings.⁴ The formulation was subsequently modified to increase cationic functionality for more efficient disruption of the lipid bilayers that comprise bacterial membranes and viral envelopes. Human coronavirus 229E (HCoV-229E), a seasonal enveloped respiratory pathogen of humans, has been employed as a surrogate for emergent coronaviruses to assess persistence, survival, and antiviral efficacy.⁵ The objective of this study was to assess the antiviral effectiveness of the new formulation following

application to stainless steel coupons against SARS-related Coronavirus 2 (SARS-CoV-2) and HCoV-229E.

METHODS

Acetone-washed, pre-autoclaved stainless-steel coupons (Type 304, unidirectional finish) coated with the reformulated quaternary ammonium polymer (SurfaceWise2) were supplied by Allied BioScience, Inc (Dallas, TX). An electrostatic sprayer mounted onto a robotic slide was used to coat the coupons, with coverage uniformity monitored by an x-ray fluorescence spectrometer. The coating was cured overnight under ambient conditions. Non-coated stainless-steel coupons were used as control surfaces. The carriers were not subjected to wear and abrasion testing prior to experimentation.

Human coronavirus 229E and the two cell lines used in the study, MRC-5 and Vero E6, were procured from the American Type Culture

Table 1

Experimental parameters for human coronavirus 229E and SARS-CoV-2 antiviral efficacy testing*

Parameter	HCoV-229E, Test 1	HCoV-229E, Test 2	SARS-CoV-2
Carrier dimensions	25.81 cm ²	6.54 cm ²	6.54 cm ²
Inoculum volume	0.10 mL	0.05 mL	0.05 mL
Soil load	None	5% FBS	5% FBS
Virus harvest method	Swab method	Wash method	Wash method
Per well assay volume	0.05 mL	0.10 mL	0.10 mL
Assay incubation period	9 days	9 days	10 days

*FBS, fetal bovine serum.

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Table 2
Viral recovery (\log_{10}) and reduction of HCoV-229E and SARS-CoV-2 on quaternary ammonium polymer-coated coupons relative to noncoated stainless steel carriers at 2 hours^{*†‡}

Coupon type	Contact time (hours)	HCoV-229E (No soil load, swab method)	HCoV-229E (5% FBS soil load, wash method)	SARS-CoV-2 (5% FBS soil load, wash method)
Noncoated control	0	4.63 ± 0.29	N.D.	5.50 ± 0.17
Noncoated control	2	4.75 ± 0.25	5.72 ± 0.25	5.06 ± 0.10
Coated test	2	≤ 0.80 ± 0.00	≤ 0.56 ± 0.10	≤ 1.53 ± 0.07
# of CPE (+) coated test carriers	N.A.	0/3	1/3	1/6
Detection limit (\log_{10})	N.A.	≤ 0.80	≤ 0.50	≤ 1.50
Log ₁₀ Reduction	N.A.	≥ 3.94	≥ 5.16	≥ 3.53
Percent Reduction	N.A.	≥ 99.98%	≥ 99.9994%	≥ 99.97%

*CPE, cytopathogenic effects; N.A., Not applicable; N.D., Not determined.

†All noncoated control and coated coupons tested in triplicate (n = 3) per contact time, except for coated test coupons against SARS-CoV-2 (n = 6).

‡FBS, fetal bovine serum.

Collection (ATCC; Manassas, VA). SARS-CoV-2 Isolate USA-WA1/2020 was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, The National Institute of Allergies and Infectious Disease (NIAID), and The National Institutes of Health (NIH). All laboratory work involving SARS-CoV-2 was conducted within a Biosafety Level 3 laboratory.

HCoV-229E (ATCC VR-740) and SARS-CoV-2 (BEI NR-52281) stocks were prepared according to the coronavirus propagation protocol described by Gundy et al. (2009)⁶ using the MRC-5 (ATCC CCL-171) and Vero E6 (ATCC CRL-1586) cell lines, respectively. Stock virus concentrations were determined using the TCID₅₀ (tissue culture infectious dose at the 50% endpoint) assay technique as described by Hierholzer and Killington (1996)⁷, and quantified using the Spearman-Kärber algorithm.

Antiviral efficacy of the coating was assessed using the American Society of Testing and Materials E1053-20 Method.⁸ Modifications to the method were 1) the use of stainless-steel coupons, and 2) preapplication of the polymer coating to the coupons followed by inoculation of virus. Test 1 against HCoV-229E (Table 1) was performed by inoculating 0.10 mL of stock virus onto polymer-coated and noncoated control coupons (25.81 cm²) mounted into sterile Petri dishes, with spreading over the surface to within ~0.32 cm of the perimeter edge. The coupons were incubated (22–23°C; 30–50% relative humidity) for 2 hours with the Petri dish lids on. Swabs predipped in Lethen Broth Base (LBB; Neogen Corp., Lansing, MI) were used for product neutralization and to harvest viruses from carriers. The swabs were placed into tubes containing 1 mL of LBB and vortexed vigorously. The suspensions were immediately passed through Sephadex G-10 gel columns by centrifugation (5 minutes at 3,500 × g). Subsequent tests against HCoV-229E (Test 2) and SARS-CoV-2 were modified by the addition of 5% soil in the form of fetal bovine serum (FBS), and by decreasing the carrier size and viral inoculum volume (Table 1). A wash method was also employed by rinsing the carriers 4–5 times using 1 mL of LBB, with further virus detachment using a cell scraper. Gel filtration followed as described. Neutralized suspensions underwent 1:10 serial dilutions using minimal essential media (Mediatech, Inc., Manassas, VA) followed by plating onto host cell monolayers in 96-well trays (6 replicates per dilution). Following incubation at 35°C in a 5% CO₂ atmosphere, wells were scored for the presence of cytopathogenic effects and cytotoxicity. The method of neutralization was also validated per ASTM E1053-20 (data not shown). Additional assay parameters are listed in Table 1.

RESULTS

The reformulated quaternary ammonium coating achieved >99.9% reduction of HCoV-229E and SARS-CoV-2 during the 2-hour

contact time in the absence and presence of 5% organic soil (Table 2). The wash method for harvesting the carriers facilitated greater recovery of viruses compared to the swab method. Cytotoxicity was observed in the lowest plated dilution (10⁰) of Vero E6 cells, while no toxic effects were evident on MRC-5 cells at the 10⁰ dilution.

DISCUSSION

The results demonstrate similar reductions of >99.9% (>3- \log_{10}) for HCoV-229E and SARS-CoV-2 following 2 hours of exposure to the reformulated quaternary ammonium coating applied to stainless steel coupons. However, further studies are warranted to assess effectiveness following application to a variety of nonporous and porous surface types. Recovery levels of both viruses from noncoated study controls after 2 hours suggest comparative rates of inactivation on Type 304 stainless steel under the environmental conditions implemented, although a larger sample size would be preferable to confirm statistical significance. Cytotoxic effects were observed after plating undiluted (10⁰) neutralized filtrates from coated carriers onto Vero E6 host cells, while none were observed on MRC-5 cells. Widespread cell monolayer damage in the absence of infectious viruses typifies cytotoxicity; therefore, dilutions that display such effects are not utilized to calculate \log_{10} reductions. However, they are determinative in establishing assay detection limits which can vary by orders of magnitude as observed for the HCoV-229E and SARS-CoV-2 assays (≤0.50 \log_{10} and ≤1.50 \log_{10} , respectively).

Surface-active coatings that have antiviral capabilities are not meant to substitute for regular cleaning and disinfection practices, but rather serve as an additional barrier for reducing human exposure to infectious viruses that may be present on fomites.⁹ With the recent detection of infectious SARS-CoV-2 from the bedside table, remote control, bed rails, and flooring in the hospital room of an infected patient,¹⁰ the importance of effective hygiene protocols for environmental surfaces remains imperative.

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