NOVEL ULTRAVIOLET LIGHT TECHNOLOGIES FOR THE INACTIVATION OF MURINE HEPATITIS VIRUS, A SARS-COV-2 SURROGATE

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Novel Ultraviolet Light Technologies for the Inactivation of Murine Hepatitis Virus, a SARS-CoV-2 surrogate

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ABSTRACT

Severe acute respiratory syndrome coronavirus (SARS-CoV-2) has infected 209 million people worldwide with millions of deaths. This virus primarily spreads through droplet contact, thus making surface transmission an ongoing concern in homes, hospitals, and commercial environments. Murine hepatitis virus (MHV) is a well-studied coronavirus that is similar to SARS-CoV-2 in its viral structure, affinity for the olfactory and pulmonary systems, neurological effects, and mode of transmission. These properties make MHV a representative surrogate virus for SARS-CoV-2 that is safer and easier to study in laboratory settings.

Short wave ultraviolet light (UV-C at 254 nm) reportedly inactivates SARS-CoV-2 particles and other pathogens on surfaces. This study compares the inactivation of the MHV surrogate using three different UV treatments on three contact surfaces. We hypothesize that a novel UV-Light emitting diode (LED) system, that is both environmentally friendly and non-toxic, will inactivate MHV on three tested model food-contact surfaces. Three UV-C systems were used in this study: a 3D UV-C system (253.7 nm) marketed to disinfect surfaces in homes; a UV-C system found in biosafety level 2 (BSL-2) hoods (254 nm); and a LED UV-C system (279 nm) that does not contain mercury and emits at a higher wavelength (279 nm).

The three surfaces used in this study included stainless steel, Formica, and ceramic. MHV (0.1 mL) at 6 log PFU/mL was spread on 3 X 3 cm^2 sterile coupons of each of these surfaces and separately treated with each of the UV-C systems for various times (with 0 min as the control). MHV was then recovered and enumerated using plaque assays on confluent L2 host cells in 6-well plates. Each experiment was carried out in duplicate and replicated thrice. Data were statistically analyzed using ANOVA (SAS v9.2). The results indicate that all three UV systems achieved at least a one-log reduction of infectious MHV within 60 seconds. The UV-C 254 nm system in the BSL-2 hood showed significantly lower D-values (causing the highest reduction within a short contact time), while the novel LED 279 nm and 3D 254 nm systems had similar D-value ranges (10-60 seconds) that varied across surface types. The novel LED UV-C system did inactivate MHV on all three surfaces thus supporting our hypothesis. However, longer surface exposure times are needed using the novel UV-LED system to obtain similar inactivation compared to the traditional UV-C 254 nm systems. This data shows promise for the potential application of UV-LED in addition to traditional UV-C to inactivate SARS-CoV-2 on surfaces.

INTRODUCTION

Severe acute respiratory syndrome coronavirus, or SARS-CoV-2, has infected 497 million people worldwide with 6.1 million confirmed deaths as of date (1). Despite the introduction of vaccines and physical measures of social distancing and wearing facemasks, this virus continues to remain a public health concern. As a respiratory virus, the primary mode of transmission is via person-to-person contact and aerosolized droplets; however, indirect transmission of the virus through fomites still poses a significant threat that is worthy of investigation to decrease the risk of outbreaks (2). Previous studies have found that SARS-CoV-2 can persist on hard surfaces, such as plastic, stainless steel, and glass, while remaining infectious between two and four days. This follows the trends of other respiratory viruses, such as human influenza virus and rhinoviruses (3). The transmission of SARS-CoV-2 through contact with surfaces can affect homes, hospitals, food environments, and other public environments. In fact, viral surveillance studies found that 50% of high-touch hospital surfaces tested positive for the presence of SARS-CoV-2 RNA, indicating the risk of fomite transmission, and highlighting the need for proper decontamination measures (4).

One proposed method for efficient, rapid, and sustainable sanitation is the use of ultraviolet (UV-C) irradiation, which is an extensively studied and widely used method of contactless viral inactivation (5). Of the three types of UV, UV-A (315-400 nm), UV-B (280-315 nm), and UV-C (100-280 nm), UV-C is most effective in its microbiocidal function (6). At 254 nm, inactivation of viral particles is caused by direct UV-C light absorption and damage to its nucleic acids, thus leading to disrupted viral replication (5). UV-C is a promising treatment for both surface contamination and aerosols (7). UV-C has been found to successfully inactivate SARS-CoV-2 particles on a variety of materials, with non-porous surfaces requiring less treatment time (8). With doses ranging from 10.25 to 23.71 mJ/cm^2, the viral titer was shown to be reduced by 99.9% (9). This suggests that UV-C could be implemented for the sterilization of a variety of surfaces that require low penetration for inactivation.

There are some disadvantages to using traditional UV-C lamps. This includes the possible leakage of mercury contained in the lamp and the warm-up time needed for the system to reach maximum power. Light emitting diode (LED) systems have been developed to emit UV-C at varying wavelengths that avoid these disadvantages. UV-LED allows for temperature-independent irradiance and immediate maximum power output, all without the use of mercury, that makes it more environmentally friendly and also sustainable with low associated costs. It can also be manipulated to emit varying wavelengths to reach maximum UV-light absorbance by DNA (260-265 nm); traditional UV-C lamps can only emit at 254 nm (6). While most of the studies have focused using traditional UV-C systems, there is limited knowledge on the use of UV-C LED systems for microbial inactivation.

This study aims to evaluate and compare the inactivation of SARS-CoV-2 using traditional UV-C lamps (254 nm) and a novel UV-LED system (279 nm). In order to compare inactivation by these systems, three model surfaces were used that are commonly found in homes, hospitals, and commercial environments, that include stainless steel (SS), ceramic, and Formica. The surrogate virus Murine Hepatitis Virus (MHV) was used in place of SARS-CoV-2, which requires biosafety level-3 containment

and not easily available in all laboratories. Murine hepatitis virus, however, can be studied in a biosafety level-2 facility. MHV has been widely used as a surrogate for SARS-CoV-2 as it is a beta-coronavirus similar to SARS-CoV-2 with a ssRNA enclosed in an enveloped capsid. The structural and genetic similarities of MHV to SARS-CoV-2 are crucial in studying viral inactivation of UV-C (10). We hypothesized that MHV will behave similar to SARS-CoV-2 and that the UV-C LED at 279 system will inactivate MHV in a similar manner to the UV-C at 254 nm system.

MATERIALS AND METHODS

Virus Propagation

MHV was propagated on in murine L2 cells, a mouse cell line from the lung epithelia. The cells were incubated at 37ºC at 5% CO2 in Dulbecco's Modified Eagle's Medium, supplemented with 8% of Fetal Bovine Serum and 1% of Penicillin-Streptomycin. MHV was propagated on confluent cells at a multiplicity of infection (MOI) of 10 in 2% Newborn Calf Serum (NCS) media (DMEM with 1% Pen-Strep). Absorption was allowed for three hours at 37ºC at 5% CO_2, then 8% NCS media (DMEM + 1% Pen-Strep) was added, making the final flask volume 20 mL. Infected cells were incubated for 48 hours at 37ºC at 5% CO_2 before freezing at -80ºC and thawing at room temperature. The freeze-thaw procedure was repeated three times. Once thawed for the third time, the contents of the flask were centrifuged at 8.0 x 1000 rpm for 15 minutes, then filtered through a 0.2 µm filter. Viral stocks were then plated on confluent 6-well plates of L2 cells to determine viral titer.

Surfaces and Treatments

Three model surfaces were used, including Formica (plastic), Stainless Steel, and Ceramic. Each surface was made into coupons with a surface area of 3 x 3 cm^2. After use, all coupons were soaked in 10% bleach for 30 minutes before being cleaned with water. Coupons were allowed to air dry before being wrapped in aluminum foil and autoclaved for reuse.

Three UV-C treatments were used, including the UV-C lamp found in a biosafety level-2 fume hood, emitting at 254 nm, a 3D-UV-C system sold commercially and marketed for home disinfection that rotates as it is emitting UV-C at 254 nm, and finally a novel light-emitting diode (LED) UV-C system that emits at 279 nm. Coupons were treated at varying distances depending on the nature of the lamp. Coupons treated in with UV-C from the BSL-2 hood lamp were placed 22 inches (55.88 cm) directly below the lamp. For the 3D UV-C system, the coupons were treated at 1 meter away from the lamp, as recommended by the lamp instructions. For the LED UV-C system, the coupons were treated directly below the system, at a distance of 3.5 inches (8.89 cm). These distances were later used, along with the lamp emittance, to calculate the dosage of each UV treatment.

Virus recovery and Enumeration by Plaque Assay:

One hundred µl of MHV stock was aseptically spread on the surface of a coupon and allowed to air dry for 10 minutes. Coupons were placed in a sterile petri dish. Once dried, coupons were then exposed to UV-C by one of three systems at varying timepoints ranging from 0 to 66 seconds (0 seconds being the control). Virus was eluted using 750 µl of 8% NCS containing media (DMEM + 1% Pen-Strep) by pipetting from the coupon 5-10 times. This liquid was then added back to the 1.5 ml dilution tube of 8% media, and then serially diluted 10-fold. These dilutions were then used to infect confluent L2 cells in 6-well plates for 3 hours, overlaid with a 1:1 mixture of 2x DMEM media and noble agar, and incubated at 37ºC at 5% CO_2 for 2-3 days. Plaques were then enumerated and recorded as PFU/mL. Each treatment was assayed in duplicated and replicated thrice.

Statistical Analysis: Statistical analysis was carried out using ANOVA (SAS v9.2) software.

RESULTS

Reduction of the infectious load of MHV was determined by subtracting the log PFU/ml of each time point of UV-C exposure time from the log PFU/ml of the 0 second control of each treatment and coupon type. *Figure 1* describes the reduction of infectious MHV across the three UV-C systems tested on Formica coupons. The UV-C system from the BSL-2 fume hood achieved the fastest reduction of MHV, taking only 5 seconds to have a one-log reduction in viral yield. The other two systems achieved similar reduction but required longer treatment times. As seen in the figure below, the LED UV-C system at 279 nm and 3D UV-C systems had lower rates of reduction, as seen through the slope of the corresponding line color. The 3D system particularly required much longer treatment times to achieve the same reduction on Formica coupons, with a one log reduction taking between 50-60 seconds.

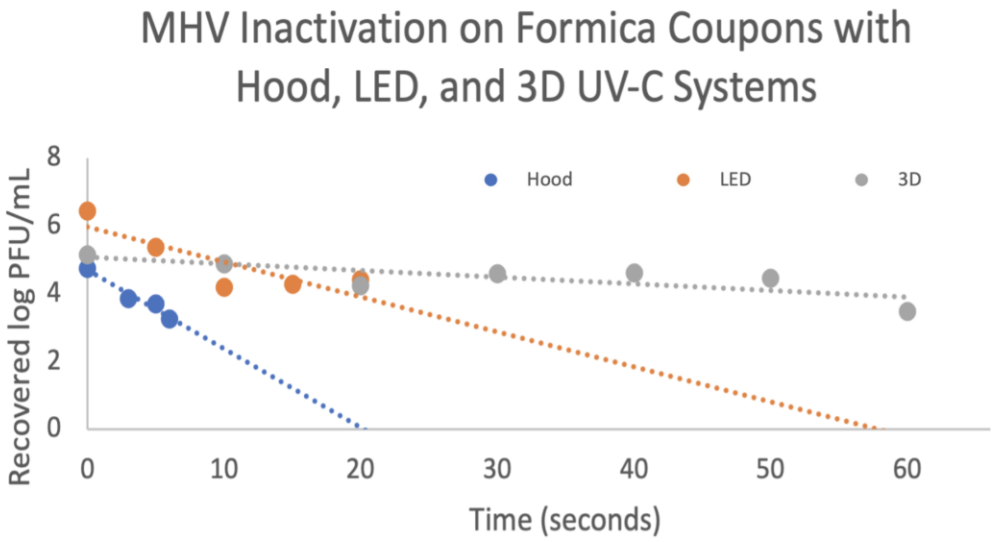

Figure 1: Reduction of MHV on Formica coupons using the BSL-2 Hood (254 nm), LED (279 nm) and 3D (254 nm) UV-C Systems

The reduction trends, however, varied between each surface type. To compare reduction more easily between each UV-C treatment and surface, we calculated and compared the D-value of each UV-C system, or the amount of time in seconds required to achieve a one-log reduction of infectious MHV yield between treated and untreated samples. These data are shown in *Figure 2*. The UV-C from the BSL-2 had the most consistent data, with this system having the lowest D-value for all three surfaces. These D-values ranged 3.52 s (Formica) to 5.45 s (stainless steel). The LED UV-C system had the largest range of D-values, with values from 10.55 s to 63.89 (stainless steel). This system did not perform as well on stainless steel coupons compared to the 3D and UV hood systems but performed competitively with the BSL-2 hood and 3D systems on both Formica and Ceramic. The 3D UV-C system also had more variance than the BSL-2 Hood UV-C, but less than the LED UV-C system. Its D-value ranges were 22.87 (stainless steel) s to 52.75 s (Formica). These data can be seen in *Table 1*. The BSL-2 Hood UV-C system consistently outperformed the other systems in regard to speed of

inactivation; however, all three systems achieved the same level of reduction in around 60 s.

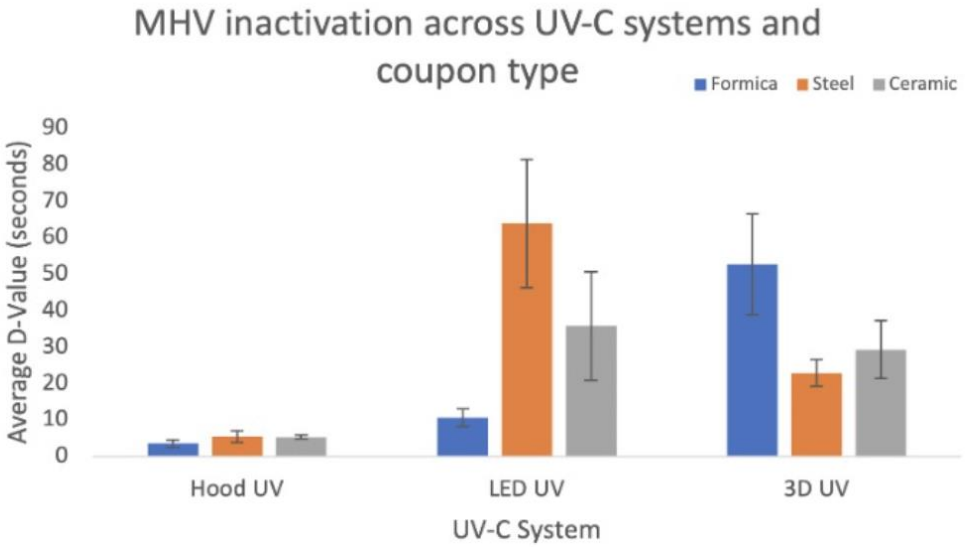

Figure 2: D-value(s) of the inactivation of MHV when treated with the BSL-2 Hood (254 nm), LED (279 nm) and 3D (254 nm) UV-C Systems on Formica, stainless steel, or ceramic coupons

Table 1 describes *Figure 2* numerically. Statistical analyses were performed on this set of data and significance is denoted in the table. There was no statistical significance across the three surfaces treated with the BSL-2 Hood UV. Across the LED UV-C system, the results with Formica and stainless steel coupons were significantly different from each other, with stainless steel having the longer D-value of 63.89 s, while the results on ceramic coupons were not significantly different from either Formica nor stainless steel. With the 3D UV-C system, again the Formica and stainless steel surfaces had significantly different results, this time with Formica having the longer D-value of 52.75 s, while the results from ceramic coupons were not significantly different from either Formica or stainless steel.

Looking at significance across the UV-C systems, with Formica coupons, the 3D UV-C system had significantly different results as compared to the BSL-2 hood and LED UV-C systems, with an average D-value of 52.75 s, as compared to 3.52 s and 10.55 s respectively. With the stainless steel surface, the LED UV-C system had significantly higher D-values than the hood and 3D UV-C systems, with a D-value of 63.89 s as compared to 5.45 s and 22.87 s respectively. Finally, across the treatment types on ceramic coupons, the BSL-2 hood UV-C system was significantly different from the LED UV-C system, while the 3D system was not significantly different from either the hood or LED UV-C system.

Table 1: D-value(s) of the inactivation of MHV when treated with the BSL-2 Hood (254 nm), LED (279 nm) and 3D (254 nm) UV-C Systems on Formica, stainless steel, or ceramic coupons *

UV system	D-Values (seconds)		
	Formica	Stainless Steel	Ceramic
Hood UV 254 nm	3.52 ± 1.0^{Ab}	5.45 ± 1.59^{Ab}	5.30 ± 0.51^{Ab}
LED UV 279 nm	10.55 ± 2.44^{Bb}	63.89 ± 17.54^{Aa}	35.82 ± 14.83^{ABa}
3D UV 253.7 nm	52.75 ± 13.86^{Aa}	22.87 ± 3.70^{Bb}	29.35 ± 7.94^{ABab}

*Capital letters denote significant differences of coupon types when compared across one UV treatment (column). Lower case letters denote significant differences when compared down UV treatments for one coupon type (row) ($p<0.05$).

These results suggest that although there is variance among surface types and UV-C treatment types, all three UV-C systems achieved reduction of infectious MHV in under ~63 seconds on all surface types. The BSL-2 hood UV-C system that emits at 254 nm achieved the lowest D-values, but it was not significantly different from the novel LED UV-C system on Formica coupons.

DISCUSSION/CONCLUSIONS

As the SARS-CoV-2 pandemic strain of coronavirus continues to remain a public health concern, new methods of surface decontamination and viral inactivation need to be investigated. The prolonged survival of infectious and viable SARS-CoV-2 particles on common surfaces found in homes, hospitals, and commercial environments is especially concerning, and methods to prevent this route of transmission are needed. With the benefits of light emitting diode (LED) short wave ultraviolet light (UV-C) systems, including being more environmentally friendly, faster acting, portable and cost efficient, as compared to traditional mercury containing UV-C lamps, it is of interest to study the use of LED UV-C systems for viral inactivation.

The results of this study indicate that the UV-C system in the BSL-2 hood achieved the most efficient viral inactivation, with the lowest range of D-values across all three surface types. Despite this, however, all three UV-C systems were able to achieve the same amount of reduction in under ~63 seconds of UV exposure. The BSL-2 hood UV-C system was also the most consistent, with no significant differences across the three surface types.

The LED UV-C system had a significantly higher D-value on stainless steel than the other two UV-C systems. Since this LED system emits at 279 nm, as compared to 254 nm emittance from the other two systems, it is the only system that targets both protein and nucleic acids. The nature of the stainless steel material in combination with the mode of action of inactivation by UV-C at 279 nm may have caused this difference. We believe the reflection of the UV-C light on the stainless steel may have had some effect on the absorbance of UV, thus affecting the inactivation of MHV. Further studies would have to be done to investigate the cause behind this increase in inactivation time on stainless steel with LED UV-C.

In addition, the nature of the 3D UV-C system may explain why its D-value ranges were higher than the UV-C from the BSL-2 hood, though it was only significantly different from the hood UV system on Formica coupons. The 3D system is designed and marketed for home use; the instructions indicate to allow the system to warm up for 5-10 minutes, place the system in the middle of a room and allow it to sterilize surrounding surfaces. Because of this, the UV system rotates from its base 180º and is designed for long-range usage. Perhaps the movement of the system decreased the overall dosage that was applied to each coupon, as compared to the stagnant LED and hood UV-C systems. This may account for the increased inactivation times needed with this system, but again, more studies would be needed to confirm this conclusion.

LED UV-C systems have the benefit of not needed a warm-up time to achieve maximum irradiance, making this system more efficient. These systems do not contain mercury, a potent biological hazard for both humans and the environment. LED can also be manipulated to emit varying wavelengths of UV-C, whereas traditional systems only emit at 254 nm. This can be particularly useful in enveloped viruses, like coronaviruses, that have surface proteins that can be disrupted at higher UV-C wavelengths, like 279 nm for example. LED is also much smaller of a system, making it portable and more cost efficient than traditional systems. With these benefits, despite needing slightly longer treatment times for similar inaction to the traditional systems, LED UV-C may be a viable option for viral inaction and surface decontamination in indoor environments.

This study showed that UV-C LED at 279 nm was capable of inactivating MHV on all the three tested contact surfaces, albeit requiring longer exposure times than the traditional UV-C at 254 nm systems for similar inactivation. Further studies on porous surfaces including cloth and wood for MHV inactivation by the UV-C LED system would provide further understanding on the application of this system for broad decontamination ability. Further work on understanding the mode of action of UV-C LED system against MHV would also provide insights for improved design of the systems and for use as hurdle approaches for optimal inactivation of respiratory viruses. In addition, calculating the UV dosage of each system regarding the distance of treatment would help make the results of this study more applicable to use in homes, hospitals and industrial environments.

ROLE IN PROJECT

My role in the project was the conducting experiments and collecting and analyzing the data. The principal idea of this project is thanks to Dr. Doris D'Souza at the University of Tennessee-Knoxville and Dr. Ankit Patras and Dr. Brahmaiah Pendyala from Tennessee State University. TSU provided the UV-C LED and 3D systems, as well as assisted with the calculations of the timepoints needed for viral inactivation. Dr. D'Souza utilized their UV-C systems and created a cell culture model to test its efficacy in her lab.

I completed all cell culture, experiments, plaque assays, and data collection independently, as well as any media preparation. I did help to optimize the experimental procedure regarding placement of coupons, treatment times, and methods of viral recovery.

Emily Camfield, M.S., statistically analyzed the data with ANOVA software. She created *Table 1* and included the significance letters. She assisted with some replicates of the data, specifically those on Ceramic coupons and LED-UV.

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