Pathogen-Repellent Plastic Warp with Built-In Hierarchical Structuring Prevents the Contamination of Surfaces with Coronaviruses

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ABSTRACT: Amidst the COVID-19 pandemic, it is evident that viral spread is mediated through several different transmission pathways. Reduction of these transmission pathways is urgently needed to control the spread of viruses between infected and susceptible individuals. Herein, we report the use of pathogen-repellent plastic wraps (RepelWrap) with engineered surface structures at multiple length scales (nanoscale to microscale) as a means of reducing the indirect contact transmission of viruses through fomites. To quantify viral repellency, we developed a touch-based viral quantification assay to mimic the interaction of a contaminated human touch with a surface through the modification of traditional viral quantification methods (viral plaque and TCID50 assays). These studies demonstrate that RepelWrap reduced contamination with an enveloped DNA virus as well as the human coronavirus 229E (HuCoV-229E) by more than 4 log 10 (>99.99%) compared to a standard commercially available polyethylene plastic wrap. In addition, RepelWrap maintained its repellent properties after repeated 300 touches and did not show an accumulation in viral titer after multiple contacts with contaminated surfaces, while increases were seen on other commonly used surfaces. These findings show the potential use of repellent surfaces in reducing viral contamination on surfaces, which could, in turn, reduce the surface-based spread and transmission.

KEYWORDS: hierarchical structures, nanostructuring, microstructure, superhydrophobic, virus-repellent

1. INTRODUCTION

The transfer of viral pathogens from a host to a healthy individual is typically broken into three mutually nonexclusive modes of transmission: airborne, droplet, and contact transmission. Airborne and droplet modes of transmission are important for perspired droplets released by the host and are classified by an aerodynamic diameter ($d_a$) smaller than 100 μm.1,2 This small size allows the droplets to stay suspended in the air and easily travel deep into the respiratory tract and interact with mucus membranes in the mouth and nose.1 Alternatively, contact transmission occurs from either direct physical contact with infected individuals (via touch, sexual contact, oral secretions, and body lesions) or indirectly through contact with contaminated surfaces.1 Surfaces may become contaminated through contact with other contaminated materials or through the deposition of large respiratory droplets and the residual solid material from an evaporated droplet.3,4 Droplets with $d_a > 100 \mu m$ settle quickly (<8 s)5 from air onto surfaces and contribute greatly to surface contamination.6 While the main transmission pathway of SARS-CoV-2 is through the contact of infected respiratory droplets with mucosal membranes,7 there is also a risk of viral transmission with contaminated hands.7,8 Furthermore, high touch surfaces (keyboards, door handles, telephones, and medical equipment) in hospital centers have shown significant contamination.8

Inanimate objects, which when contaminated, assist in the transfer of infectious materials to a new host are known as fomites, and they play a key role in the spread and transmission of viruses and pathogenic infections via direct and indirect contacts.1,9 The indirect contamination pathway of viruses commonly occurs between fomites and hands (Figure 1a) and can effectively transfer up to 65% of the virus concentration from the fomite to uncontaminated hands.9 Contaminated hands can then transfer the virus to the body through the mouth, nose, or eyes.2,9 In addition to spreading the virus to a new host, fomites also increase the lifetime of viruses outside a
host, contributing to increased overall viral transmission rates. Pathogens from the coronavirus family (SARS-CoV-2, Middle East respiratory syndrome (MERS), and human coronaviruses (HuCoV)) have been shown to remain infective for up to 9 days on common surface materials such as metal, glass or plastic. In particular, human coronavirus 229E (HuCoV-229E), in low titers (10³ pfu/mL and 10² pfu/mL), stays active and infective for up to 5 days¹¹ and 4 days¹² respectively, when deposited within organic matter/debris (human lung cell debris, in human feces),¹¹,¹³ implying that the contaminated organic matter/debris can increase the lifetime and survivability of a virus outside the host.

Engineered surfaces that prevent or greatly reduce contamination when exposed to viruses show great promise in mitigating the spread of viruses via indirect contact transfer. Antiviral coatings made from disinfectants (ethanol, glutaraldehyde, hydrogen peroxide, sodium hypochlorite)¹⁴ or engineered materials (copper, zinc, silver, TiO₂, N,N-dimethylhexadecylamine)¹⁵ are commonly used to reduce the viral titer on high touch surfaces. These reagents and/or materials are either harmful to the surface (hydrogen peroxide),¹⁰ to the individuals touching them (peracetic acid),¹¹ or to the environment (sodium hypochlorite),¹¹,¹₂ or present a delay, in the order of hours (>4 h) between contamination and disinfection (TiO₂, copper),¹³ leading to the spread of viruses during such long transition times.

An alternative rapid and safe way of halting the surface-mediated transmission of viruses involves the use of liquid-repellent surfaces (repelling high- and low-surface-tension fluids) that reduce virus binding onto surfaces, the formation of fomites, and contamination of surfaces with potentially contaminated droplets. Two classes of liquid-repellent surfaces, liquid-infused²⁵⁻²⁹ and hierarchically structured surfaces,³⁰⁻³⁴ have shown to significantly repel contaminating liquids while also preventing the adhesion of pathogens and formation of biofilms at the surface. Liquid-infused surfaces integrate a lubricating liquid into a porous or rough surface using the chemical affinity that exists between the surface and the lubricant, causing contaminants to slide away from the surface, resulting in self-cleaning properties.³⁵ Lubricant-infused surfaces significantly reduce the attachment and growth of bacteria and their biofilms by over 90% over the course of multiple days.³⁶,³⁷ Introduction of antipathogenic materials, such as carvacrol and antibiotics, into the lubricant has also been utilized to reducing the transmissibility of bacteria (Staphylococcus aureus and Pseudomonas aeruginosa), fungi (Bacillus subtilis), and viruses (Zika Virus) by nearly 100%.³⁸,³⁹ Lubricant-infused surfaces have also displayed self-healing properties through the swelling of the surface structure in the presence of the lubricant;⁴⁰ however, the number of healing cycles is limited by the amount of lubricant trapped in the surfaces.³⁸ Despite high performance in liquid²⁷,⁴¹ and pathogen²⁵,³⁶,³⁸,⁴² repellency, liquid-infused surfaces are
difficult to operate in open-air conditions due to the evaporation of the liquid layer and the limited stability of such surfaces. On the other hand, hierarchically structured surfaces do not rely on liquid infusion and provide liquid-repellent properties by reducing the apparent surface energy through the formation of unique wetting states by trapping air pockets within their surface structure (Cassie state). This effect is enhanced through chemical modification with hydrocarbon and fluorocarbon chains, improving repellency with low-surface-tension liquids and resulting in liquid-repellent properties.

Hierarchically structured surfaces with an extremely high liquid repellency have demonstrated significant pathogen repellency. Among these surfaces, a hierarchically structured surface with a diamond architecture, created through hot filament chemical vapor deposition, reduced the adherence of P. aeruginosa and Escherichia coli on the surface by >90%. Similar performance has also been achieved through the use of photoablation and photolithographic techniques to achieve >89 and 99% reduction in the adhesion of E. coli, respectively. In addition to bacterial repellency, reduced adhesion of adenoviruses HAdv4 (2.10 log) and HAdv7a (1.62 log) has also been demonstrated with superhydrophobic textiles modified with fluorinated polytetrafluoroethylene nanoparticles. While having high performance, many of these surfaces are fabricated using difficult-to-scale and expensive methods such as photolithography, chemical vapor deposition, or photoablation. In response, we have developed a facile and scalable method, based on all-solution-processing and heat shrinking, to create hierarchically structured surfaces on flexible substrates. These surfaces decreased the formation of biofilms of pathogenic Gram-negative (P. aeruginosa) and -positive (methicillin-resistant Staph aureus (MRSA)) bacteria by ~85% and demonstrated a 20-fold decrease in bacterial (E. coli) adhesion. These surfaces also have a significant repellency toward biological liquids (whole blood CA 144 ± 2° and fetal bovine serum CA 158.3 ± 2.6°), which can mediate the transmission of pathogens. Recent studies have also displayed superhydrophobic surfaces having a significant reduction in viral contamination when interacting with virus-laden droplets. These surfaces can significantly reduce the contamination from airborne droplets during sneezing or the interaction of the surface with bodily fluids. However, these studies have only looked at the interaction of surfaces with the
liquid phase and data is still missing on how liquid-repellent surfaces interact under direct contact (i.e., surface-to-surface) transmission of viruses.

In this work, we evaluate a hierarchically structured superhydrophobic flexible plastic that has been previously shown to repel low- and high-surface-energy liquids and reduce the adhesion of bacteria and formation of biofilms,54,55 in its ability to reduce the adhesion of viruses. To model the transfer of viruses between surfaces, we developed a touch assay coupled with viral quantification to detect the presence of active viral particles transferred to surfaces with high sensitivity. To establish the assay, we first used an enveloped virus, herpes simplex virus 2 (HSV-2) with a similar size as SARS-CoV-2, and we then used HuCov-229E, a coronavirus from the same family as SARS-CoV-2, to evaluate the viral repellency of the engineered surfaces. Our experiments demonstrate that such engineered surfaces, when placed on regular surfaces, can greatly reduce the level of viral contamination.

2. RESULTS AND DISCUSSION

2.1. RepelWrap Fabrication and Quantification of Surface Repellency and Durability. The repellent plastic wrap (RepelWrap) was created by coating the surface of a shrinkable plastic wrap with an aminosilane linker (APTES) for binding the plastic to the nanoparticle layer, depositing a layer of silica nanoparticles (SiNPs) as a stiff layer needed for wrinkle generation, and adding a molecular layer of fluorosilanes as the hydrophobic chemistry (Figure 1b). Following the coating of the plastic wrap with the desired materials, it was heat-shrunk to form hierarchical micro/nanoscale wrinkles decorated with SiNP (Figure 1c,ii).34,51,54,55

The RepelWrap combines a unique physical structure, microscale wrinkles with SiNP building blocks, with chemical functionalization for achieving repellency.34

To quantify the repellent properties of RepelWrap, we measured its static contact angle (CA) and contact angle hysteresis (CAH) and compared these metrics to other materials (polyethylene and aluminum) commonly used in high touch surfaces such as handles, railings, hand tools, and countertops. CA is a measure of the wettabillity of the surface and is used to assess the surface free energy,66 whereas CAH measures the difference between advancing and receding contact angles and is used to assess the activation energy required for a droplet to slide on the surface.57 RepelWrap showed superior hydrophobicity (water CA:153 ± 3.6°) compared to both polyethylene and aluminum (water CA:104 ± 3.7 and 94 ± 1.8°, respectively; Figure 2a). The CA of the surfaces was also analyzed using hexadecane, glycerol, and ethylene glycol to provide insight into different liquid surface interactions and to measure their level of wettabillity to the surface. Hexadecane in particular has a significantly lower surface tension than water (27.47 vs 72 mN/m) and only interacts with itself and surfaces through dispersive forces, unlike water that interacts with surfaces through dipole–dipole interactions and hydrogen bonding.58

These properties make hexadecane more efficient at wetting and contaminating surfaces and make it suitable for quantifying the molecular interaction of dispersive forces at a surface. RepelWrap displayed superior performance in repelling hexadecane (hexadecane CA: 124 ± 1.6°) compared to the control surfaces (hexadecane CA: polyethylene 35 ± 7.2° and aluminum 10 ± 2.6°). RepelWrap showed superior CA of 153.6 ± 1.3° for glycerol and 141.6 ± 2.7° for ethylene glycol, compared to the control surfaces of aluminum (glycerol CA: 42.0 ± 1.9° and ethylene glycol CA: 24.8 ± 1.3°) and polyethylene (glycerol CA: 92.9 ± 3.6° and ethylene glycol CA: 83.2 ± 3.7°). Additionally, RepelWrap exhibited much lower CAH with various liquids in comparison to the control surfaces (Figure 2a). A sliding angle of <5° (5 μL) was also seen on the RepelWrap for both water and glycerol; however, sliding was not observed with ethylene glycol and hexadecane due to pinning of the liquid droplets into the surface structure. Sliding angle is a measure of droplet mobility59 and strongly correlates with CAH, with low sliding angles being strongly correlated to repellency.60 The strong repellency of all of the test liquids (all CA > 90° and CAH < 20°) observed here is the result of the formation of Cassie–Baxter wetting states on the micro- and nanoscale features of the surface (Figure 1ci), which are not present on the planar surfaces of polyethylene and aluminum (Figure 1ciii,iii).34 To confirm that all solutions were in the Cassie–Baxter wetting state on the surface of the RepelWrap, the fractional wetting of the surface was calculated for both water (0.122) and hexadecane (0.299). Values of fractional wetting smaller than one, as observed here, represent Cassie–Baxter wetting.61 Furthermore, modulation of the SiNP size (27, 100, and 200 nm diameters) was used to tune the scale of the micro- and nanostructures (Figure S3). Comparison of the three surfaces showed that the increase in scale results in a decrease in the CA (Figure S3d) and an increase in the CAH (Figure S3e) for all probing liquids. This ability to control the wettabillity for all probing liquids also allows for the easy tunability of the surface wetting for relevant applications. Furthermore, it was previously determined that a significant portion of the hexadecane performance was due to the presence of fluorine groups (fluorosilane) on the surface, making the combination of the physical structure and the chemical modification essential for repellency.34

To determine the stability and resiliency of RepelWrap, we re-evaluated the repellent properties of the surfaces before and after repeated contact with both elastomeric stamps and a human finger (Figure 2c,d). For the elastomeric stamp experiments, the water contact angle of the surfaces was measured every 30 stamps, each with a duration of 10 s and a force of 697 ± 1 mN, up to a total of 90 stamps. There was no measurable change in the hydrophobicity (~150° CA and >5° CAH) and oleophobicity (~120° CA and >15° CAH for hexadecane) of the surfaces upon repeated stamping, demonstrating the robustness of RepelWrap (Figure 2c). For the finger touch experiment, the surfaces were pressed with a force of 10 793 ± 1213 mN for roughly 1 s, with a time delay of 1–2 s between the touches. The contact angle was measured after every 150 touches, and no significant change in repellency of water (~150° CA and >5° CAH) or hexadecane (~120° CA and >15° CAH) was observed for the maximum number of touches that was assessed (Figure 2d), further demonstrating the resiliency of the surfaces against frequent usage on high touch surfaces (Figure 2b). The shear stability of the surface was also quantified to better understand how forces parallel to the surface will impact the wettabillity. After shearing of the surface with 200 and 500 g weights, no significant change was seen in the wettabillity of the surface with water. From Figure S5, it is also clear that the effects of shearing have a larger influence on the wetting of hexadecane, as greater variation in the contact angle measurements was observed for all shear cases compared to that of the control. The sustained contact
(>150°) and sliding angle (<10°) of water have been well reported for hierarchical wrinkled structures for various deformations (stretching, bending, and torsion) as well as abrasion and compression of the surface. We have also previously shown that it is possible to cover objects with different form factors using the shrinking process involved in the fabrication of RepelWrap. The surface structure after both repeated stamping and touching with a finger was also investigated using SEM (Figure S3). These images show slight damage/crushing on the peak of the hierarchical wrinkle structure; however, the internal deeper re-entrant structure of the wrinkles, which is mainly responsible for the formation and stability of Cassie–Baxter wetting states, remains intact. Similar structural damage to hierarchical wrinkled microstructures has been observed in the past, which was paralleled with a decrease in the water contact angle from 160 to 147°.

2.2. Evaluation of RepelWrap in Reducing Viral Contamination Using an Enveloped Virus (HSV-2) as a Model. Once it was determined that the RepelWrap remains repellent following frequent touching, as is the case with high touch surfaces, we sought to determine whether the liquid-repellent properties of the RepelWrap (high contact angle with various liquids and low sliding angle) can be translated to viral repellency (Figure 3). For this purpose, viral quantification was performed for HSV-2 (diameter of ~125 nm). This pathogen was chosen because it contained a phospholipid envelope and had a similar size as SARS-CoV-2 (diameter of 60–140 nm), allowing it to be used as a surrogate model to evaluate the potential applicability of RepelWrap against the spread of COVID-19. The assay was performed by first contaminating an elastomeric stamp with a solution of Dulbecco’s minimum Eagle’s medium (DMEM) containing the virus (1.33 × 10^6 pfu/mL), then stamping the surfaces with the stamp to mimic contamination with the human touch, and finally incubating the contaminated surface with cell growth media used in the viral quantification assay (Figure 3a). It is important to note that DMEM has a similar viscosity (0.94 CP) and CA (152.3 ± 3°) on RepelWrap as water, while the CAH of DMEM differs significantly from water (35 ± 5°). This indicates that interactions between DMEM droplets on contaminated surfaces should have slightly higher wetting and attractive properties compared to water droplets during the contact transmission of viruses. The virus titer present in the media was quantified using the vero-cell plaque assay (Figure 3b), in which the number of developed viral plaques were counted for different serial dilution factors (10-fold) and used to calculate the viral titer. The potential antiviral properties of the fluorsilane molecule and surface coating were also investigated (in solution and on planar surfaces) to ensure that the reduced surface contamination of viruses was due to the repellent properties of the hierarchical wrinkled structure. These control experiments demonstrated no measurable decreases in the viral titer in the presence of fluorsilane, indicating the importance of the hierarchical structuring of RepelWrap in viral repellency (Figure S1). It is evident from the abovementioned assay that RepelWrap is far less susceptible to becoming contaminated after contact, showing more than 4-logs reduction in HSV-2 titer compared to that of aluminum and polyethylene (Figure 3b).

2.3. RepelWrap Significantly Reduces Contamination with Coronaviruses. Given the remarkable ability of RepelWrap in reducing contamination with HSV-2, we sought to answer whether it would also be possible to reduce contamination caused by HuCoV-229E (diameter of 80–120 nm), a coronavirus from the same family as SARS-CoV-2, which is responsible for the COVID-19 pandemic. As such, the abovementioned stamping assay was used to contaminate RepelWrap and control surfaces with HuCoV-229E, and the viral titer transferred to the surfaces was quantified using an endpoint dilution assay in which the serial dilution at which 50% cell lysis occurred was used to calculate the initial viral titer, via the Reed–Muench method. Similar to the HSV-2 experiment, RepelWrap reduced the viral titer by 4-logs compared to aluminum and polyethylene, confirming its high performance in repelling viruses. To further analyze the ability of the RepelWrap in reducing contamination on high touch surfaces, a multitouch version of the assay was performed using HuCov-229E, in which the surfaces were stamped three times.
consecutive times (each with a viral titer of $9.80 \times 10^5$ TCID$_{50}$/mL) with 1 min intervals between stampings. The comparison of the single- and multitouch experiments shows a 0.33- and 0.16-log increase in contamination on the polyethylene and aluminum (Figure 4a), respectively, after the two additional touches. Remarkably repeated contact with a contaminated surface causes no increase in the viral titer measured on the RepelWrap. This, in conjunction with the maintained liquid repellency ($\sim 150^\circ$) after repeated physical contact (Figure 2c,d), shows that the repellent properties sustain after repeated exposure to both contamination and mechanical strain.

The ability of RepelWrap in repelling contamination is further confirmed by electron microscopy (Figure 4b). Scanning electron microscopy shows clusters of viruses adhered to both polyethylene and aluminum; however, no visible contamination is observed across the RepelWrap (Figure 4b). To further confirm that the contamination seen in Figure 4b is in fact the viral particles (and not crystallized culture solution), control surfaces were contaminated with culture solution and without the virus (Figure S4). Unlike the virus-contaminated surfaces, the particles of crystallized culture media on the control surface were much more sparsely distributed across the surface and much larger in diameter (500 nm to 5 μm) than the viral particles (diameter $\sim 100$ nm) seen in Figure 4b. These findings are in strong agreement with the viral quantification assay and highlight the superior performance of RepelWrap for repelling viruses when coming in contact with fomites.

3. CONCLUSIONS

In this work, a contact transfer viral quantification assay was developed to investigate the interaction of viruses with RepelWrap, a highly repellent hierarchically structured surface. The quantification of the viral contamination was conducted with both DNA (HSV) and RNA (HuCov-229E) viruses. RepelWrap repelled various liquids (water CA 153° and hexadecane CA 124°) and maintained its repellent properties after repeated physical contact with both elastomeric stamps and a human finger. When brought into physical contact with stamps contaminated with viruses, RepelWrap showed little to no contamination with HSV-2 or HuCov-229E, while other materials commonly used in high touch surfaces (aluminum and polyethylene) showed significant contamination. These repellent properties were retained after repeated contact with contaminated stamps, whereas the common surfaces displayed an increase in the amount of contamination.

We attribute the virus repellency of RepelWrap to the reduced interaction of virus-containing water droplets with the surface, reduced interaction of viral capsid proteins and biomolecules with the surface, or a combination of both. It is possible that moisture mediates the suspension and transfer of viral particles to high touch surfaces as such, superhydrophobic surfaces that are highly effective in repelling water are less likely to get contaminated. Alternatively, reduced
interaction/adsortion of viral capsid proteins in the presence of superhydrophobic wetting states could reduce the adhesion of viruses from stamps to RepelWrap. Similar mechanisms have also been used to explain the reduced formation of biofilms and blood clots on hydrophobic surfaces, given that protein adsorption is the first step in these processes. The adsorption of proteins to a surface is strongly related to the chemical properties, surface charge, surface structure, and the hydrophilicity/hydrophobicity of the surface, the most significant of these effects are the long-range electrostatic interactions and hydrophobic effects, and as such, the unique properties of the RepelWrap could attribute to reduce protein adhesion. More research is needed to better understand the exact mechanisms at play for the interaction of viruses with these classes of surfaces. A better understanding of these interactions may also help us to develop new classes of surfaces for preventing the spread and survivability of pathogens on surfaces.

4. METHODS

4.1. Reagents. (3-Aminopropyl)triethoxysilane (APTES, 99%), 1H1H2H2,2H2F-perfluorodecyltriethoxysilane (fluorosilane, 97%), and Ludox TMA colloidal silica were purchased from Sigma-Aldrich (Oakville, Ontario). Ethanol (anhydrous) was purchased from Green Field Global (Mississauga, Ontario). Polydimethylsiloxane, elastomer and curing agent were purchased from Ellsworth Adhesives (Stoney Creek, Ontario). Hydrochloric acid (36.5–38%) was purchased from Caledon (Georgetown, Ontario). Milli-Q grade water (18.2 MΩ cm) was used to prepare all solutions. HSV-2 thymidine kinase-deficient (TK2/-) HSV-2 ATCC, vero-cell line Vero (ATCC CCL-81), and human coronavirus 229E (ATCC VR-740) were purchased from ATCC (Manassas, Virginia). Huh7.5 cells were kindly provided by Dr. Charles M. Rice (Rockefeller University, New York City, NY).

4.2. Surface Fabrication. Polyolefin (Cryovac D-955) was cut into 15 cm disks and subsequently cleaned with ethanol and Milli-Q water and dried with compressed air. The polyolefin substrates were then exposed to air-plasma in an expanded plasma cleaner (Harrick Plasma) on HIGH RF power for 1 min. The surfaces were then contaminated by pipetting 20 μL of DMEM + 10% FBS with a viral load of 1 × 10^7 pfu/mL onto the stamp; this was then followed by placing a glass coverslip on the contaminated side of the stamp to evenly disperse the solution across the surfaces and left for 10 min. After removal of the glass slide, the stamps were left until the remaining excess solution was adsorbed or evaporated from the now contaminated surface. Before stamping, the high touch surface models were made by adding 2 μL of DMSO to 30 μL of each viral dilution to ensure no contaminants were present on the surfaces. The virus-contaminated stamps were then placed, contaminated side down, onto the surfaces for 1 min with a 70 g weight. The stamps were then removed, and the surfaces were placed into a 12-well plate containing 400 μL DMEM + 10%FBS. This solution was then used for titration.

4.3. Surface Physics Characterization. SEM imaging was performed on a JEOL 7000F. Samples were coated with 5 nm of platinum before imaging. Contact angle, contact angle hysteresis, and sliding angle measurements were taken on a KRUS SDA30S Drop Shape Analyzer (Hamburg, Germany) with droplets of water (5 μL) and hexadecane (5 μL).

4.4. Durability to Physical Contact Test. Contact angle and contact angle hysteresis were measured on RepelWrap with a KRUS SDA30S drop shape analyzer (Hamburg, Germany) to characterize the wettability of the surfaces. PDMS stamps and human fingers were repeatedly brought into contact with the hierarchically structured surfaces for a given number of contacts (30 and 150 for the PDMS stamp and human finger, respectively). This process was then repeated several times for each surface.

4.5. PDMS Stamp Fabrication. Sylgard 184 silicone elastomer base and elastomer curing agent were mixed in a ratio of 10:1. Once fully mixed, the elastomer was cured overnight in an oven at 60 °C. The elastomer was then cut into their final shape, 1 cm x 1 cm cubes, and sonicated in ethanol for 10 min followed by drying with compressed air.

4.6. Cell Cultures. Human hepatoma cells, Huh7.5, were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Life Technologies Inc.), 100 μg mL⁻¹ penicillin, 100 mg mL⁻¹ streptomycin (Sigma-Aldrich, Oakville, ON, Canada), 10 μM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (McMaster University), and 2 μg mL⁻¹-glutamine (L-glut) (Life Technologies Inc.). Experiments were performed with cells at passages 2–6. All cells were incubated at 37 °C in a humidified 5% CO₂ incubator. Vero cells (ATCC) were cultured in minimum essential medium Eagle-alpha modification (α-MEM) media supplemented with 5% FBS in addition to all of the additives mentioned above for DMEM.

4.7. Virus Contact Transfer Assay. The stamping surface was contaminated by pipetting 20 μL of DMEM + 10% FBS with a viral load of 1 × 10^7 pfu/mL onto the stamp; this was then followed by placing a glass coverslip on the contaminated side of the stamp to evenly disperse the solution across the surfaces and left for 10 min. After removal of the glass slide, the stamps were left until the remaining excess solution was adsorbed or evaporated from the now contaminated surface. Before stamping, the high touch surface models were made by adding 2 μL of DMSO to 30 μL of each viral dilution to ensure no contaminants were present on the surfaces. The virus-contaminated stamps were then placed, contaminated side down, onto the surfaces for 1 min with a 70 g weight. The stamps were then removed, and the surfaces were placed into a 12-well plate containing 400 μL DMEM + 10%FBS. This solution was then used for titration.

4.8. HSV-2 Titration (Plaque assay). A vero-cell plaque assay, previously described elsewhere, was used to quantify the virus concentration on the touch-transferred surfaces.

4.9. huCoV-229e Titration (TCID50). Huh7.5 cells in a 96-well plate at a seeding density of 5 × 10^4 cells per well were cultured in DMEM 10% FBS. The cells were then left in the incubator until 100% confluency was reached. The virus-containing solution was then serumized in 10% FBS, and 10 μL of each viral dilution was added to the corresponding well of confluent cells, starting at the highest dilution. The plate was left for 2 h at 37 °C in a 5% CO₂ incubator and was shaken every 15 min. Then, 150 μL of DMEM and 10% FBS solution were added to each well. The cells were incubated for 6 days and finally stained using crystal violet solution. The Reed and Muench method was used for the calculation of the virus titer.

4.10. Viral Infectivity of Fluorosilane. Fluorosilane solutions were made by adding 2 μL of DMSO to 30 μL of fluorosilane stock (30%). The solutions were diluted using a-MEM 0%. The fluorosilane surfaces were manufactured using the protocol discussed above. A 60 μL viral solution was added to the fluorosilane solutions. These were then incubated for 20 min before the virus concentration was quantified. In the surface assays, 6 μL of the viral solution was added to the surfaces and incubated for 20 min. These surfaces were then washed with cell media, and viral concentration was quantified. The initial viral titer (HSV-2) of 10^6 pfu/mL was used for both the fluorosilane solutions and surfaces. This experiment was repeated two times, and the results were consistent.

4.11. Scanning Electron Microscopy: Virus Fixation. The HuCoV-229E were stamped onto the polymer and metallic surfaces as described in the previous section. The stamped surfaces were then incubated in 4% paraformaldehyde (PFA) (EM grade) in phosphate-buffered saline (PBS) to fix the viruses. Then, surfaces were rinsed with Milli-Q water and air-dried and afterward coated with 3 nm of platinum for imaging using a JEOL 7000F (JEOL, Peabody).
Quantification of fluorosilane viral inactivation properties, analysis of surface structure partial wetting with liquids of different surface tensions, structural comparison of various surfaces manufactured with various size nanoparticles with corresponding SEM images and wettability with various liquids, SEM images of the surface structure after repeated stamping and contact with a human finger, SEM images of polyethylene surfaces after stamping with uncontaminated culture media, and surface structure wettability after shearing with various masses (PDF).

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**Author Contributions**

V.R.M. and F.V. contributed equally to this work. The design of this study was performed by R.M., S.M.I., F.V., A.A.A., T.F.D., and L.S. The experimental data were acquired by R.M. and F.V. R.M. wrote the initial draft of the manuscript, which was critically read and edited by S.M.I., F.V., A.A.A., T.F.D., and L.S. All authors have given approval for the final version of the manuscript.

**Notes**

The authors declare no competing financial interest.

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