

Antiviral Nanostructured Surfaces Reduce the Viability of SARS-CoV-2

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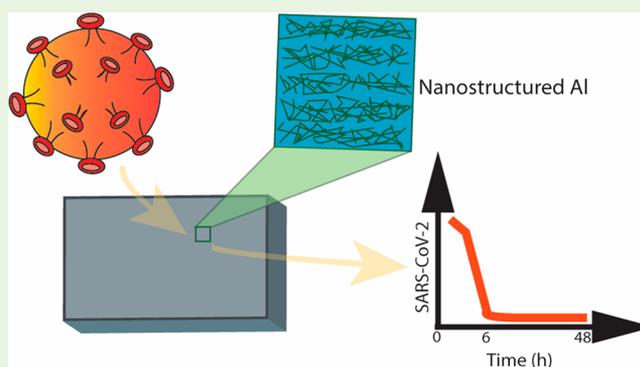
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ABSTRACT: In this letter, we report the ability of the nanostructured aluminum Al 6063 alloy surfaces to inactivate the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). There was no recoverable viable virus after 6 h of exposure to the nanostructured surface, elucidating a 5-log reduction compared to a flat Al 6063 surface. The nanostructured surfaces were fabricated using wet-etching techniques which generated nanotextured, randomly aligned ridges approximately 23 nm wide on the Al 6063 alloy surfaces. In addition to the excellent mechanical resilience properties previously shown, the etched surfaces have also demonstrated superior corrosion resistance compared to the control surfaces. Such nanostructured surfaces have the potential to be used in healthcare environment such as hospitals and public spaces to reduce the surface transmission of SARS-CoV-2 and combat COVID-19.

KEYWORDS: nanostructured surfaces, antiviral surfaces, SARS-CoV-2, nanoscale topography



INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the current global COVID-19 pandemic, with approximately 15 million global cases of infections at date. This novel human coronavirus is highly contagious and transmitted by aerosols and surfaces contaminated by fomites. Viruses, including SARS-CoV-2, can remain active on inanimate surfaces for several days.^{1–3} Although the mode of transmission of SARS-CoV-2 is not completely known, it has been found that surface contamination plays a major role as a possible route of transmission.⁴

There are currently no specific therapies or vaccines against SARS-CoV-2, which makes it difficult to contain the spread of the virus. We are therefore dependent on environmental transmission controls and public health interventions to slow the spread of infection. Chemical-based surface disinfectants such as sodium hypochlorite, hydrogen peroxide, ethanol, benzalkonium chloride, and chlorhexidine digluconate are differentially effective and require reapplication and reuse.³ Therefore, novel technologies such as nanoscale surface texturing,⁵ that are both effective at inactivating the virus and easily applied, are required to combat COVID-19 and effectively prevent the spread of SARS-CoV-2 and future virus outbreaks.

In this paper, the efficacy of the nanostructured metal surfaces against SARS-CoV-2 has been reported for the first

time. We have evaluated the antiviral performance against SARS-CoV-2 of nanostructured Al 6063 alloy surface generated through wet-etching, compared to smooth aluminum surfaces and the tissue culture polystyrene plates.

MATERIALS AND METHODS

Fabrication. Aluminum 6063 alloy sheets of 2.5 mm thickness were obtained commercially from Alsun Aluminum, Brisbane, Australia, and cut into discs of 10 mm diameter using water-jet cutting at the Design and Manufacturing Centre, QUT. Nanostructured surface topography on Al surfaces was fabricated using a wet-etching technique with 2 M NaOH for 3 h, as described previously.⁵ Unetched Al 6063 discs were used as smooth controls.

Surface Characterization. Scanning electron microscope (TESCAN MIRA3) was used to visualize the surface morphology of the etched surfaces. The SEM was operated at 10 keV using a secondary electron detector. The lateral dimensions of the nanostructures were measured using the inbuilt software (TESCAN MIRA3).

Corrosion Experiments. Electrochemical experiments were conducted on control and nanostructured Al surfaces using a three-

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electrode cell containing phosphate buffered saline (PBS, a biological solution) solution at room temperature by means of a VMP3-based BioLogic instrument controlled by EC-Lab software. The Al discs were subjected to electrochemical impedance spectroscopy (EIS) and linear polarization resistance (LPR) measurements. A platinum sheet was used as counter electrode, a standard Ag/AgCl (VL_Ionode004) as reference electrode with the sample of interest as working electrode.

EIS measurements were obtained using a frequency range of 100 kHz to 100 mHz with an amplitude of ± 10 mV. ZFit analysis of EC-Lab software was used to fit impedance spectra. LPR measurements were run at a potential scan rate of 0.1 mV/s and potential range of -25 to $+25$ mV. LPR plots were analyzed using the polarization resistance (R_p) fit tool of EC-Lab software to calculate the R_p , corrosion current density (i_{corr}), and corrosion potential (E_{corr}) values within ± 20 mV from the corrosion potential.

Viral Viability Investigation. SARS-CoV-2 stock (strain QLD02/2020, GISAID accession number EPI_ISL_407896) was propagated in Vero E6 cells (ATCC, C1008, CRL-1586, Manassas, USA) as previously described.⁶

Smooth control disks, nanostructured etched Al alloys, and polystyrene tissue culture plate (TCP) surfaces were exposed to 10 μL of viral inoculum (1×10^5 TCID₅₀/mL) and incubated at room temperature (BSC-2) for the time points 1, 3, 6, 24, and 48 h. Discs were exposed in three independent experiments. To recover live virus, at each respective time point, the exposed surfaces were washed twice by gently pipetting up and down with 32 μL of Opti-MEM reduced serum growth medium (ThermoFisher Scientific, Life technologies, Australia Pty Ltd.) 10 times (total volume 64 μL). Live, infectious SARS-CoV-2 stock and subsequent viral suspensions recovered from experimental surfaces were titrated using serial 10-fold dilutions (8 replicates of each) in 96-well microtiter culture plates seeded with confluent monolayers of Vero E6 cells. Plates were incubated at 37 °C for 7 days and 50% tissue-culture infectious dose per milliliter (TCID₅₀/mL) scores were calculated according to observed cytopathic effect and the Reed–Meunsh algorithm.

RESULTS AND DISCUSSION

Randomly oriented nanostructures were generated on the Al 6063 alloy surfaces upon etching. SEM revealed depth variations between ridges, making the surfaces appear rough with root-mean-squared roughness of 995 ± 114.7 nm compared to 0.6 ± 0.1 nm on control surfaces, confirmed through atomic force microscopy over a $1 \mu\text{m} \times 1 \mu\text{m}$ scanning area.⁵ The nanostructures on the etched surfaces had a width of $23 \text{ nm} \pm 2 \text{ nm}$, where they were grouped randomly in the form of parallel ridges, visible at high magnifications (Figure 1). This corresponded to nanostructures we had generated for previous studies.⁵ We found that the etched surfaces were hydrophilic with a static water contact angle

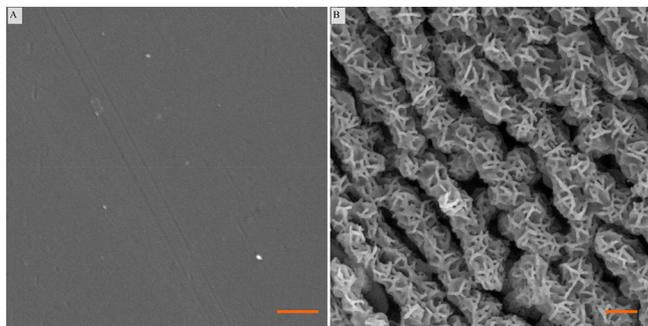


Figure 1. Scanning electron micrographs of the (A) smooth control (scale bar = 1 μm) and (B) etched Al (scale bar = 200 nm) with nanostructured topography.

(WCA) of $17.7^\circ \pm 4.3^\circ$, compared to $96.3^\circ \pm 2.3^\circ$ on the control surface.⁵

We have previously demonstrated that wet-etched Al 6063 possesses excellent antibacterial properties against Gram-positive and Gram-negative bacteria.⁵ In the same study, the surfaces were also found to be antiviral against respiratory syncytial virus (RSV) and rhinovirus (RV), in that live infectious virus was reduced within hours of exposure. Here, we have shown for the first time the effect of nanostructured topography against SARS-CoV-2. Since the virus is known to remain active for different time intervals on various surfaces, the viability of the virus was examined over a period of 1–48 h. It was found that upon exposure to the etched surface, the viability of the virus was reduced significantly within 6 h ($t = 0.014$; $0.01 < p < 0.05$) compared to exposure to smooth Al control and TCP surfaces (Figure 2). With a 5-log reduction,

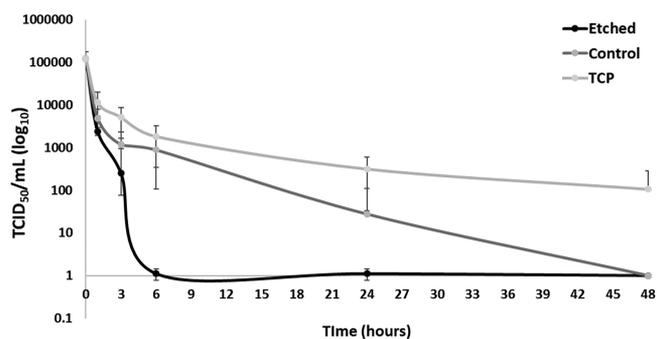


Figure 2. Viability of SARS-CoV-2 on the surfaces of the etched (nanostructured) Al 6063 alloy, control Al 6063 alloy and nonmetal surface tissue culture plates (TCP) at different time intervals of 1, 3, 6, 24, and 48 h. The titers of viable viruses are expressed as TCID₅₀/mL in logarithmic scale.

there was practically no live virus recovered from the etched surfaces at 6 h or later hours of exposure. Reduced viability on etched surfaces was also observed after 3 h, as viable virus was recovered from 1 of the 8 discs used, although was not significantly different from the control surfaces. Interestingly, on the polystyrene TCP surface, SARS-CoV-2 remained infectious for 48 h; however, there was around 2.5-log reduction in viability. This is similar to another study in which SARS-CoV-2 remained viable for up to 72 h on plastics and stainless steel.⁷

In comparison, the virus was no longer viable on the smooth aluminum surface at 48 h, with 3–4 log reduction observed after 24 h of exposure. The inactivation of virus by untreated aluminum is therefore not as effective as copper, which is a naturally antimicrobial material, inactivating SARS-CoV-2 within 4 h and SARS-CoV within 8 h.⁷

This is a novel finding which demonstrates the efficacy of nanostructured Al 6063 alloy in reducing viable SARS-CoV-2 on surfaces, thus reducing the risk of transmission by fomite contamination. We have previously demonstrated that wet-etched nanostructured Al 6063 reduces the viability of respiratory viruses, respiratory syncytial virus (RSV) and rhinovirus (RV-16). Viability of RSV and RV-16 exposed to the nanostructured Al 6063 was reduced at 6 and 24 h postexposure. The mechanism of viral inactivation by wet-etched nanostructures remains to be elucidated, although our data would suggest that viruses differ in their susceptibility. This may potentially be due to size and trapping within the

nanostructures or the composition of the viral envelope or capsid. Further work is required to understand this mechanism.

Nanoscale topography that is inspired by insect wing surfaces can be achieved on a number of materials using varying fabrication methods.^{8–12} Our data show that nanostructured surface topography provides an effective preventive technology against the spread of respiratory viruses and in particular, transmission of SARS-CoV-2 via environmental contamination. However, in order to be effective in a hospital setting, etched metal surfaces need to be durable. We have previously tested the mechanical and physicochemical surface characteristics of smooth and etched Al 6063 and found that the nanostructures rendered this material both hydrophilic, and durable against large shear and normal forces.⁵ In order to confirm if the etched surfaces can withstand corrosion and chemical breakdown, we measured corrosion resistance of the surfaces (EIS and LPR measurements). The nanostructured Al sample demonstrated a significantly higher polarization resistance (R_p) of (13538 ohms) compared to the smooth Al sample (537 ohms) (Table 1). The slope of the curve was

Table 1. Data Extracted from the Electrochemical Experiments

	R_p (Ω)	E_{corr} (mV)	i_{corr} (μA)
control	540	-635	48.53
etched	13500	-659	1.92

significantly (>20) higher for the smooth surface compared to the etched surface, indicating that the smooth surfaces had a lower R_p and a greater corrosion rate (Figure 3). Moreover, this phenomenon was also found to be consistent with the EIS measurements where the nanostructured Al sample showed

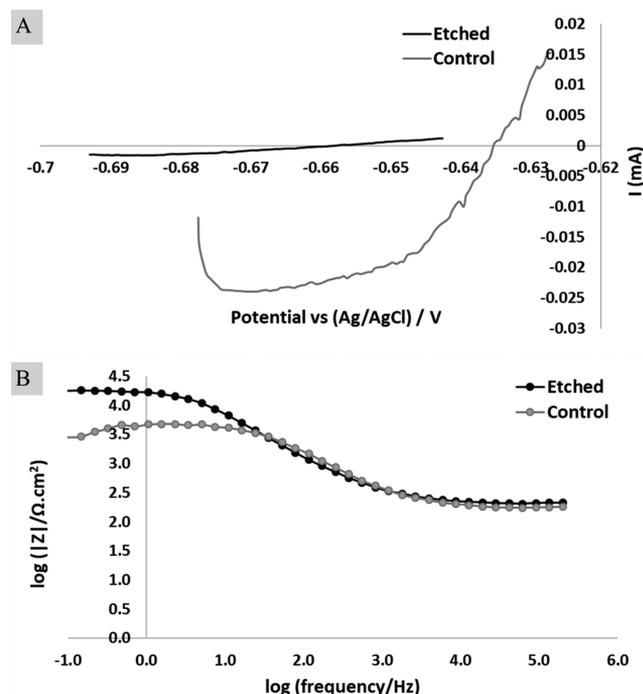


Figure 3. (A) Linear polarization resistance (LPR) curves of the etched and control samples and (B) impedance spectra (EIS) of etched and control samples in PBS solution showing Bode impedance magnitude plot.

significantly higher impedance at low frequency (Figure 3B). Overall, the nanostructured etched surfaces showed an enhanced anticorrosion potential than the control counterpart in the presence of a buffered saline solution which attests to its efficacy in high-use environments such as hospital surfaces. Al 6063 is commonly used for furniture, appliances, architectural extrusions, door frames, window panels, kitchen equipment, and hospital and medical equipment.¹³ Therefore, it would be feasible to apply the etched surfaces of the alloy to impart antimicrobial behavior on the areas of hospitals that are at high risk for transmission such as hospital trolleys and bedrails.

CONCLUSIONS

We have successfully produced durable antiviral surfaces that inactivates SARS-CoV-2 within 6 h. The results provide evidence that nanostructured surfaces are effective in preventing SARS-CoV-2 and the subsequent environmental spread. Installation of such surfaces in healthcare environments is a viable strategy for the reduction in nosocomial infections and environmental contamination and thus affects the route of transmission. Generation of nanostructures on different materials and understanding the antiviral mechanism will provide further developments to fully address the issues around viral spread.

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Notes

The authors declare no competing financial interest.

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