RESEARCH ARTICLE

Rapid inactivation of SARS-CoV-2 by titanium dioxide surface coating [version 2; peer review: 2 approved]

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Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission occurs via airborne droplets and surface contamination. Titanium dioxide (TiO₂) coating of surfaces is a promising infection control measure, though to date has not been tested against SARS-CoV-2.

Methods: Virus stability was evaluated on TiO₂⁻ and TiO₂⁻Ag (Ti:Ag atomic ratio 1:0.04)-coated 45 x 45 mm ceramic tiles. After coating the tiles were stored for 2–4 months before use. We tested the stability of both SARS-CoV-2 Spike pseudotyped virions based on a lentiviral system, as well as fully infectious SARS-CoV-2 virus. For the former, tile surfaces were inoculated with SARS-CoV-2 spike pseudotyped HIV-1 luciferase virus. At intervals virus was recovered from surfaces and target cells infected. For live virus, after illuminating tiles for 0–300 min virus was recovered from surfaces followed by infection of Vero E6 cells. % of infected cells was determined by flow cytometry detecting SARS-CoV-2 nucleocapsid protein 24 h post-infection.

Results: After 1 h illumination the pseudotyped viral titre was decreased by four orders of magnitude. There was no significant difference between the TiO₂ and TiO₂⁻Ag coatings. Light alone had no significant effect on viral viability. For live SARS-CoV-2, virus was already significantly inactivated on the TiO₂ surfaces after 20 min illumination. After 5 h no detectable active virus remained. Significantly, SARS-CoV-2 on the untreated surface was still fully infectious at 5 h post-addition of virus. Overall, tiles coated with TiO₂ 120 days previously were able to inactivate SARS-CoV-2 under ambient indoor lighting with 87% reduction in titres at 1 h and complete loss by 5 h exposure.

Conclusions: In the context of emerging viral variants with increased transmissibility, TiO₂ coatings could be an important tool in containing
SARS-CoV-2, particularly in health care facilities where nosocomial infection rates are high.

**Keywords**
Surface, SARS-CoV-2, coating, spray, TiO2
Amendments from Version 1

We have amended the intro, added details to methods and discussion but not results.

Any further responses from the reviewers can be found at the end of the article

Background

Respiratory droplets are believed to be the major vehicle of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission. Droplets or other body fluids from infected individuals can contaminate surfaces and viable virus has been detected on such surfaces, including surgical masks, for hours, even days depending on different factors including humidity, temperature and type of surface. One therefore infers that any external contamination of personal protective equipment (PPE) may last hours or even days.

Recently, there has been a further increase in SARS-CoV-2 cases globally, despite severe mitigation measures following the first wave in the first half of 2020. The new UK, Brazilian and South African variants (S01Y.V1/V2/V3) have led to global anxiety and high levels of nosocomial transmission within hospitals are being observed in the 2020/21 UK winter despite universal adoption of wearing face masks, regular testing of staff and patients, and social distancing measures. It may well be that contamination of surfaces is now disproportionally contributing to transmission.

Traditional forms of decontamination (such as alcohol-based sprays, quaternary ammonium compounds, and sodium hypochlorite and other chlorine-based compounds) require repeated applications. Photocatalytic surfaces, on the other hand, permanently oxidize, inactivate and destroy microorganisms under normal ambient lighting conditions. A recent hospital study of titanium dioxide-coated surfaces demonstrated progressive lowering of the bacterial biofilm. Moreover, the radicals are not considered to induce antimicrobial resistance. TiO₂ is especially attractive because it is considered nontoxic to humans: titanium, coated with its oxide, is the most widely used material for implants. TiO₂ is also exceedingly stable, unlike other photocatalysts such as zinc oxide and tungsten trioxide. Illumination of TiO₂ generates highly oxidizing free radicals that are known to have bactericidal and antiviral action against influenza and rotavirus. SARS-CoV-2 has not hitherto been investigated.

Methods

Cell lines

293T and Vero E6 cells were cultured in DMEM complete (DMEM, Sigma D5030) supplemented with 100 U/ml penicillin (Sigma), 0.1 mg/ml streptomycin (Sigma), and 10% fetal calf serum, Gibco, (ThermoFisher). Vero E6 were a gift from Prof. Ian Goodfellow. 293T cells were a gift from Prof Greg Towers. ACE-2/TMPRSS2-expressing 293T cells were generated by transfecting plasmids expressing ACE-2/TMPRSS2 from a CMV promoter in pCDNA3.1 (ThermoFisher Cat no: V79020). pCAGGS-SARS-CoV-2 Spike expressing plasmid (NIBSC cat no: 100976), 1ug p8.91HIV-1 gag-pol expression plasmid (a gift from Prof Greg Towers) and 1.5ug pCSFLW (expressing the firefly luciferase reporter gene with the HIV-1 packaging signal – a gift from Prof Greg Towers)44. Plasmids were mixed in OptiMEM (GIBCO, Thermofisher Cat no: 31985062) Following transfection in 10cm plastic petri dishes (Nunc cat no:150464), viral supernatant was collected at 48 and 72 h after transfection, filtered through a 0.45 μm filter (Millipore, cat no: HAWP04700) and stored at ~80 °C. The 50% tissue culture infectious dose TCID₅₀ of SARS-CoV-2 pseudovirus was determined using the Steady-Glo luciferase (Promega cat no: E2550) assay system including a luminometer (Glomax Navigator Lumimeter, Promega, cat no: GM2000).

Viral isolate

Live SARS-CoV-2 (SARS-CoV-2/human/Liverpool/REMRQ0001/2020) used in this study was isolated by Lance Turtle (University of Liverpool), David Matthews and Andrew Davidson (University of Bristol). A SARS-CoV-2 virus stock was produced by infecting Vero E6 cells at MOI 0.01. Culture supernatant was collected 48 h post-infection. The titre of the stock was determined by adding tenfold serial dilutions of virus onto Vero E6 cells. 24 h post-infection cells were fixed by removing media and replacing with 3% paraformaldehyde in PBS. Samples were stained for nucleocapsid protein using a monoclonal rabbit anti-Nucleocapsid antibody (1:1000, MA5-36086, ThermoFisher) and % infection determined by flow cytometry on a BD FACSCalibur instrument, with 10,000 cells counted. SARS-CoV-2 virus titres were determined as infectious units per ml (IU/ml) as follows: (% infected cells) × (total number of cells) × (dilution factor) / volume of inoculum added to cells.

Surfaces and illumination

Ceramic tiles were wiped down with neutral disinfectant then coated with either a TiO₂ based solution or with a combination of TiO₂ and Ag using a spray gun (bespoke). The tiles were allowed to dry for 15 minutes. The spray gun was connected to an 11L air compressor (Makita). The nozzle orifice was 8 microns with pressure fixed at 12 PSI to atomise the coating. Virus stability was evaluated on the following surfaces: sterile untreated Sterilin standard Petri dish; TiO₂ and TiO₂–Ag (Ti:Ag atomic ratio 1:0.04)-coated 45 × 45 mm ceramic tiles (Invisi Smart Technologies UK Ltd). The coatings are transparent and colourless and therefore invisible to the human eye. After coating the tiles were stored for 2–4 months before use. Surfaces were exposed (610 lx, ambient temperature 21 °C (in a microbiological safety cabinet).

Surface inoculation and sampling

SARS-CoV-2 spike pseudotyped virus inactivation. Tile surfaces were inoculated with 10⁵ RLU of SARS-CoV-2 spike pseudotyped HIV-1 luciferase virus onto the surface of the tiles at a dosage of 5 μl over 5 × 5 mm at time t = 0 and illuminated for up to 6 h. Three individual 5x5mm spots were used on the same tile. At...
SARS-CoV-2 live virus inactivation. 6×10⁶ IU/ml of SARS-CoV-2 virus was added onto the surface of the tiles at a dosage of 2 μl over 5 × 5 mm. Three individual 5x5mm spots were used on the same tile. After illuminating for 0–300 min virus was recovered from surfaces with 50uL of DMEM complete followed by infection of Vero E6 cells. % of infected cells was determined by flow cytometry detecting SARS-CoV-2 nucleocapsid protein 24 h post-infection.

Kinetic analysis of inactivation

The main challenge is that laboratory inactivation experiments are necessarily carried out with large numbers of viruses, with which the inactivating material is brought into contact at the beginning of the experiment, and the decay of the entire virus population is measured[15]. What is of practical interest in the scenario of a coating designed to keep surfaces (e.g., in a hospital) free of viral (and bacterial) bioburden is how quickly an individual virus is inactivated. According to analysis of previously reported results for influenza virus inactivation[11], the kinetics fit a convective diffusion transport model even in the absence of mechanical agitation, most likely due to almost inevitable thermal gradients[15]. The concentration of survivors is thereby predicted to follow a so-called exponential decrease, and plotting the logarithm of the number of survivors v. time should give a straight line, the slope of which is –k, the inactivation rate coefficient. The value of k can then be compared with the transport-limited fastest possible rate calculated from the size of the virus[15].

Statistical analysis

We did not perform statistical analyses in this work.

Results

After 1 h illumination the pseudotyped viral titre was decreased by four orders of magnitude (Figure 1A[16]). There was no significant difference between the TiO₂ and TiO₂–Ag coatings. Light alone had no significant effect on viral viability.

Figure 1. Effect of TiO₂/Ag and TiO₂ coated tiles on. (A) SARS-CoV-2 Spike pseudotype viral infection and (B) SARS-CoV-2 isolate REMQ0001/Human/2020/Liverpool (C) Plot of the experimental data from (B) as ln(%infected cells) v. time (s). Dashed line represents a linear regression using the first 4 data points. The slope of the line yields a disinfection rate coefficient k.
Next, we tested the ability of the coated tiles to inhibit fully infectious live virus. Coated and uncoated surfaces were exposed to SARS-CoV-2. Virus was harvested at the times indicated and used to infect Vero E6 target cells. SARS-CoV-2 was already significantly inactivated on the TiO₂ surfaces after 20 min illumination. After 5 h no detectable active virus remained (Figure 1B). Significantly, SARS-CoV-2 on the untreated surface was still fully infectious at 5 h post-addition of virus. TiO₂–Ag appeared somewhat less effective than TiO₂ alone, but the difference was not significant.

Plotting the experimental data (Figure 1B) as ln(titre) v. time (Figure 1C) yields a disinfection rate coefficient k of 5.2 ± 0.6 × 10⁻³ s⁻¹, which corresponds to the transport-limited fastest possible rate estimated for SARS-CoV-2 approaching a disinfecting surface in water. Hence we infer that the viruses arriving at the surface from the inoculum are essentially immediately inactivated. From our illumination conditions we estimate the generation rate of radicals as about 10¹³ cm⁻² s⁻¹, corresponding to about 800 radicals s⁻¹ over the area occupied by one virus at the surface. By extrapolating the data from the first four points to the assumed detection limit, it can be seen that very likely no detectable virus from the initial inoculum remained soon after 2 h exposure (Figure 1C).

**Discussion**

The potent extended anti-SARS-CoV-2 effect of titanium dioxide surface coatings is highly desirable in hospital settings where both patients and staff might be shedding viruses. An important advantage of these surfaces is that they can be activated by ordinary interior light and do not need UV irradiation, which is usually incompatible with simultaneous human presence. The coating has a rough surface with high local curvature that creates an absorption tail into the blue region of the visible spectrum, overlapping the spectral output of ordinary interior lighting. This is sufficient to ensure an adequate rate of radical generation for effectively immediately inactivating viruses and other microorganisms arriving from the air or hand touches. Conversely, a limitation is that if a sudden very large contamination event occurred, particularly one that severely diminished the light reaching the photocatalyst, it might take impractically long for the contamination to be eliminated.

Hence, in that case rough cleaning, even washing with water, should be used to remove the gross contamination. Further limitations to our study include the lack of a control tile that was unsprayed for the virus isolation work, and the fact that activity against droplets from infected individuals may be different as a result of other material such as mucus present. Controlled experiments with respiratory secretions would not be feasible.

The efficacy of the TiO₂ coating under typical hospital lighting makes it a promising candidate for enhancing the protection afforded by facemasks and other PPE, and well as surfaces likely to be contaminated and hence acting as reservoirs for transmitting infection if left untreated. The coatings would need only periodic re-application depending on the amount of wear, for example 6-12 monthly, and would be widely available in the future. Such interventions are increasingly critical in conditions where viral variants with increased transmissibility are the new norm.

**Data availability**

**Underlying data**

DRYAD: SARS-CoV-2 viability after exposure to titanium dioxide coated tiles. https://doi.org/10.5061/dryad.4j0zpc89z16

This project contains the following underlying data:

- PM_luminometer_data-1.xlsx (Luminometer data for SARS-CoV-2 spike pseudotyped virus inactivation)
- TiO2_fcs_files.zip (Flow cytometry data for SARS-CoV-2 live virus inactivation)

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

**Acknowledgment**

The authors thank Saba Yussouf (Invisi Smart Technologies) for helpful discussions. We would also like to thank Nigel Temperton for the plasmids.

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Version 2

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✔ Gert U. van Zyl
1 National Health Laboratory Service, Tygerberg Hospital, Cape Town, South Africa
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The authors have adequately addressed all my minor concerns.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Diagnostic and clinical virology, HIV persistence, HIV drug resistance, viral epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 13 September 2021
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✔ Xuan Xue
School of Pharmacy, University of Nottingham, Nottingham, UK

I have no further comments to make.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antiviral materials, surface chemistry, polymer chemistry, biomaterials, high
throughput screening

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 26 July 2021

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? Xuan Xue
School of Pharmacy, University of Nottingham, Nottingham, UK

In this research article, the authors demonstrated that titanium dioxide (TiO$_2$-) and TiO$_2$-Ag coating of surfaces can be a promising measure to reduce the fomite transmission of SARS-CoV-2 for future infection protection control. TiO$_2$ has been previously demonstrated to have bactericidal and antiviral action against influenza and rotavirus, but not yet studied on SARS-CoV-2, by generating oxidizing free radicals under light. This research compared the stability of SARS-CoV-2 Spike pseudo virus and infectious SARS-CoV-2 virus on coated and uncoated surfaces, and the results of which evidenced that TiO$_2$- and TiO$_2$-Ag coatings, compared to the uncoated control surfaces, effectively inactivated both SARS-CoV-2 pseudo and live viruses under ambient indoor light within hours. The authors indicated the potential of TiO$_2$ coatings as a tool in contribution to the control of SARS-CoV-2 transmission in public and health care settings. In this manuscript, the work has been well designed, clearly presented and concluded with relative adequate data.

Some minor comments are stated below:

1. There are a few different types of materials, e.g. metal-based, silicon-based, carbon-based, and polymers, that have been showing antiviral potentials. It would be good to briefly review the current literature on them and indicate the merit for investigating more on TiO$_2$-coatings than others.

2. The coating procedure was described in the methods section without further optimisation. Also, there are no further discussions on the validation and evaluation of the successful coatings on the surface. A series of surface characterisation techniques are therefore recommended, for example, ToF-SIMS and XPS to confirm the surface chemistries and their distributions after coating, and SEM to show the surface features and uniformity of the coating.

3. The virus stability was evaluated on both TiO$_2$-coated and uncoated (control) surfaces. According to Fig. 1 B, polystyrene surface was used as the control substrate. However, as the coatings were applied on tiles (please specify its material) and no data have evidenced
the successful coating (please see Comment 2), an uncoated tile surface would be a more appropriate control substrate compared to polystyrene.

4. In order to recommend on effective inactivation of virus on TiO$_2$-coated surfaces for health care facilities in the future, it would be good to have more details of the light applied in this research as well as investigate more on different lights, in terms of their type, power, and applied distance to surfaces, in this study.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
Not applicable

**Are all the source data underlying the results available to ensure full reproducibility?**
Partly

**Are the conclusions drawn adequately supported by the results?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Antiviral materials, surface chemistry, polymer chemistry, biomaterials, high throughput screening

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

**Author Response 07 Sep 2021**

**Ravindra Gupta**, University of Cambridge, Cambridge, UK

Reviewer 2:
In this research article, the authors demonstrated that titanium dioxide (TiO$_2$)- and TiO$_2$-Ag coating of surfaces can be a promising measure to reduce the fomite transmission of SARS-CoV-2 for future infection protection control. TiO$_2$ has been previously demonstrated to have bactericidal and antiviral action against influenza and rotavirus, but not yet studied on SARS-CoV-2, by generating oxidizing free radicals under light. This research compared the stability of SARS-CoV-2 Spike pseudo virus and infectious SARS-CoV-2 virus on coated and uncoated surfaces, and the results of which evidenced that TiO$_2$- and TiO$_2$-Ag coatings,
compared to the uncoated control surfaces, effectively inactivated both SARS-CoV-2 pseudo and live viruses under ambient indoor light within hours. The authors indicated the potential of TiO<sub>2</sub> coatings as a tool in contribution to the control of SARS-CoV-2 transmission in public and health care settings. In this manuscript, the work has been well designed, clearly presented and concluded with relative adequate data.

Some minor comments are stated below:

1. There are a few different types of materials, e.g. metal-based, silicon-based, carbon-based, and polymers, that have been showing antiviral potentials. It would be good to briefly review the current literature on them and indicate the merit for investigating more on TiO<sub>2</sub>-coatings than others.

Response: A formal review of literature would be lengthy. Hence we have focused on the tile-based TiO<sub>2</sub> coating.

1. The coating procedure was described in the methods section without further optimisation. Also, there are no further discussions on the validation and evaluation of the successful coatings on the surface. A series of surface characterisation techniques are therefore recommended, for example, ToF-SIMS and XPS to confirm the surface chemistries and their distributions after coating, and SEM to show the surface features and uniformity of the coating.

Response: Unfortunately these experiments are beyond the scope of this report but are important as the reviewer points out.

**Competing Interests:** none
manuscript is overall well-constructed, accessible and well-written.

I have a few minor comments for improvement of overall clarity:

1. Please clarify how the photocatalytic activity of TiO$_2$ is affected by natural vs incandescent vs fluorescent light bulbs?

2. Please describe the time intervals and procedure of sampling to assess infectivity in detail (were different spots sampled on the same surface?)

3. Please clarify the nature of the control surface used. The text referred to an “uncoated surface” which I assumed would be ceramic, but the figure refers to polystyrene (is the latter an appropriate control surface?)

4. Considering the potential of this surface coating: Please clarify a) how frequently the coating would have to be reapplied, b) what the cost of coating per square meter would be and c) how widely available TiO$_2$ is for use as a spray for surface coating.

5. Please comment on the difference of respiratory droplets that contain mucous vs free cultured virus and how this may have affected the inactivation of viral particles.

Is the work clearly and accurately presented and does it cite the current literature? 
Yes

Is the study design appropriate and is the work technically sound? 
Yes

Are sufficient details of methods and analysis provided to allow replication by others? 
Partly

If applicable, is the statistical analysis and its interpretation appropriate? 
Not applicable

Are all the source data underlying the results available to ensure full reproducibility? 
Partly

Are the conclusions drawn adequately supported by the results? 
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Diagnostic and clinical virology, HIV persistence, HIV drug resistance, viral epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
Ravindra Gupta, University of Cambridge, Cambridge, UK

Reviewer 1:
Micochova et al. described the inactivation of SAR-CoV-2 on surfaces coated with titanium dioxide (TiO$_2$). TiO$_2$ is photocatalytic under ambient light, producing oxidative radicals that inactivate infectious agents. The inactivation of SARS-CoV-2 pseudotyped virus and live SARS-CoV-2 cultures were assessed on ceramic tiles coated with either TiO$_2$ or TiO$_2$-silver(Ag) by culturing viruses from these surface at different time intervals. The authors found that after 5 hours, no infective virus remained on the treated surface but it was still fully infectious on the untreated surface.
The manuscript adds valuable novel data and provides an apparently practicable solution to coating surfaces in hospitals, which would result in surface disinfection under ambient light. The manuscript is overall well-constructed, accessible and well-written.

I have a few minor comments for improvement of overall clarity:

1. Please clarify how the photocatalytic activity of TiO$_2$ is affected by natural vs incandescent vs fluorescent light bulbs?

Response: Both natural (i.e. sunlight) and incandescent filament lamps approximate to black body radiators, albeit with different temperatures. The photocatalytic activity, which depends on the convolution of the incident light with the absorption spectrum of the TiO$_2$, is therefore qualitatively the same for the two. The degree of activity (as measured e.g. by rate of inactivation of viruses) will simply depend on the actual irradiances -- to a first approximation linearly. Fluorescent lighting has a different spectral distribution. For a good estimate of the degree of photocatalytic activity, one can simply take the irradiance in the near UV-violet-blue spectral range and compare it with that of the natural or incandescent source.

1. Please describe the time intervals and procedure of sampling to assess infectivity in detail (were different spots sampled on the same surface?)

This has now been described in the methods in more detail

1. Please clarify the nature of the control surface used. The text referred to an “uncoated surface” which I assumed would be ceramic, but the figure refers to polystyrene (is the latter an appropriate control surface?)

Response: We used plastic as a control surface in the absence of an uncoated tile. We have now stated this in the limitations section.

1. Considering the potential of this surface coating: Please clarify a) how frequently the coating would have to be reapplied, b) what the cost of coating per square meter would be and c) how widely available TiO$_2$ is for use as a spray for surface coating.

Response: We have now specified in the paper that the coating would be re-applied every 6-12 months. The frequency depends on the adhesion of the coating and the degree of wear to which it is subjected. With no wear the coating lasts indefinitely. With moderate hand-touching the coatings used in this work will typically last 6-12 months.
The cost is a few dollars per sq m and the coating is planned to be made widely available. The last two comments cannot really be put in the text as this is a changing area

1. Please comment on the difference of respiratory droplets that contain mucous vs free
cultured virus and how this may have affected the inactivation of viral particles. Response: Mucous mainly contains mucin, a glycoprotein family, which would be photocatalytically oxidized to CO2 and H2O by TiO2. Partially oxidized intermediates may themselves be radicals that would damage and inactivate the viruses. Without detailed mechanistic investigations it is difficult to predict whether the mucous accelerates or retards virus inactivation. The ultimate outcome is not expected to be different, however. We have now commented on this in the discussion and thank the reviewer for this point.

**Competing Interests:** No competing interests were disclosed.