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Title: Antiviral activity of salt-coated materials against SARS-CoV-2.

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Abstract

The SARS-CoV-2 pandemic demonstrated the importance of human coronaviruses and the need to develop materials to prevent the spread of emergent respiratory viruses. Coating of surfaces with antiviral materials is a major interest in controlling spread of viruses, especially in high risk or high traffic areas. A number of different coating for surfaces have been proposed, each with their own advantages and disadvantages. Here we show that simple salt coating on a range of surfaces, including a novel biomass aerogel can reduce the infectivity of SARS-CoV-2 placed onto the surface. This suggests that a simple to apply coating could be applied to a range of materials and have an antiviral effect against SARS-CoV-2, as well as other potential emerging viruses.

Introduction

Human coronaviruses (hCoVs) are important human pathogens, but until recently have not caused significant disruption to society. hCoVs can be broadly grouped into seasonal and emerging hCoVs. The seasonal hCoVs, such as hCoV-299E and hCoV-OC43, usually cause mild 'common-cold-like' disease in healthy adults, but can occasionally cause significant outbreaks in settings with vulnerable populations, such as nursing homes (for example: 1). Prior to 2020 there were two emerging hCoVs described: severe acute respiratory syndrome (SARS)-CoV-1 (previously known as SARS-CoV) and Middle East respiratory syndrome (MERS)-CoV. Both SARS-CoV-1 and MERS-CoV are zoonotic viruses that caused significant disease outbreaks, with high case fatality rates, but were (and, in the case of MERS-CoV, still are) geographically restricted For MERS-CoV this is, in part, due to the distribution of the zoonotic source, the dromedary camel (*Camelus dromedarius*). The intermediate host for SARS-CoV-1 was the masked palm civet (*Paguma larvata*), which is not as geographically restricted, but was successfully eliminated from humans primarily through effective quarantine of infected individuals (2). MERS-CoV continues to

cause human infections, but is primarily a camel virus (3) and cannot spread between humans easily under normal conditions (4). Therefore, neither SARS-CoV-1 nor MERS-CoV have reached pandemic level.

The current ongoing outbreak of SARS-CoV-2, however, has demonstrated the pandemic potential of coronaviruses emerging into the human population as hCoVs causing 694 million confirmed cases and 6.9 million deaths, as of August, 2023, while spreading to nearly every country and continent in the world, including Antarctica (https://www.bbc.co.uk/news/world-europe-59848160).

Human coronaviruses have been recognised as a significant cause of common-cold-like illnesses since the 1960s, but despite the emergence of SARS-CoV-1, had not been seen as having major pandemic potential. Therefore, upon the emergence of SARS-CoV-2 we were not equipped with the tools needed to combat SARS-CoV-2. Despite significant advances in developing effective vaccines and new anti-viral drugs against SARS-CoV-2, the constant emergence of new variants, waning immunity in vaccinated populations and drug side effects mean that personal protective equipment and biosecurity measures continue to play a major role in providing population level protection against any new outbreaks. Hence, there is an urgent need to improve the efficacy of existing measures such as antiviral surfaces or face masks to prevent the spread of SARS-CoV-2 and future respiratory virus outbreaks.

The use of a variety of face coverings was one of the most widely adopted SARS-CoV-2 mitigation policies, despite considerable controversy as to the efficacy of specific policies (5, 6). The properties of each mask, including material, fit to the face and filtration capacity can have a big impact on their efficacy (7, 8). However, face masks coated with simple antiviral materials could be an important tool to prevent the spread of any virus. This is particularly the case when there is a novel virus, such as SARS-CoV-2, for which it will take some time to develop effective vaccines or drugs. An effective face mask may prevent the critical early spread of the virus and decrease viral load even if not eliminating exposure, effectively cutting off transmission at a time when the infection is still at low enough level to be effectively managed and/or controlled.

Previous studies have coated materials with various coatings and many have shown antiviral effects (reviewed in 9). But, salt coating of various materials has been proposed as an effective tool to prevent the spread of respiratory pathogens (10) and they have previously been shown to be anti-bacterial against a range of important human pathogens (11). Additionally, salt coating of surfaces can be antiviral, both *in vitro* and, interestingly, *in vivo* (12, 13). Specifically, coating surfaces prevented the spread of influenza viruses by inactivating viruses that passed through the coated filter (13) and reduced the stability of a pig coronavirus, transmissible gastroenteritis virus (12).

Here we describe a number of simple, cost effective and easily scalable materials that show anti-viral activity against SARS-CoV-2 and could be rapidly deployed to prevent transmission in high-risk environments. The antiviral efficacy of coated surface was initially demonstrated using

an animal orthobunyavirus namely Schmallenberg virus (SBV) which is not pathogenic in humans and can readily replicate in Vero E6 cells, the cells used for the SARS-CoV-2 work and, therefore, was a more readily reproducible surrogate for SARS-CoV-2.

Initial testing of antiviral efficacy of materials is typically done with a lower pathogenicity surrogate enveloped virus for safety reasons, in this case an animal orthobunyavirus, which is not pathogenic for humans, Schmallenberg virus (SBV) was used. A selection of coatings with high anti SBV activity were then tested for their ability of neutralise SARS-CoV-2.

Materials and methods

Materials.

A full list of materials and coatings tested is provided in Supplementary Material 1 and 2.

Table salt was purchased from Tesco PLC. Sodium chloride and potassium chloride were purchased from Fisher Scientific. Sodium percarbonate was purchased from Amazon. Biomass aerogels (Figure 1) were developed and provided from the Hubei University of Technology (14). Surgical mask was provided by KMD Company Ltd.

Non-sterile 12cm by 10cm pieces of non-woven fabrics and melt-blown fabrics (Figure 1) were soaked in the 200 mL of each salt solution and then dried in a 50^{o}C drying oven. A small humidifier was used to spray 5 mL salt water onto the surface of non-sterile 15cm^{2} pieces of biomass aerogels and then dried with a hairdryer. For the facemask (KMD Company limited), the inner and outer layers are non-woven fabric and the middle layer is melt-blown fabric. A similar biomass aerogel to the one used in this study has been previously reported (15, 16).

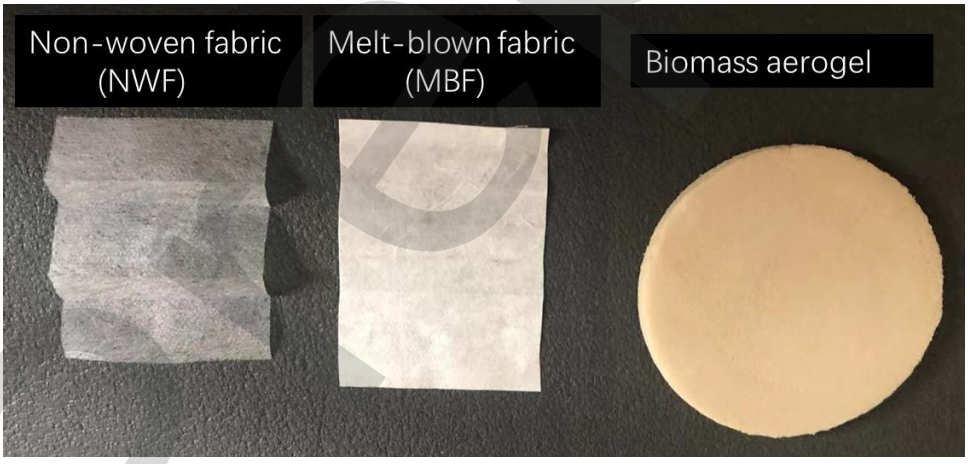

Figure 1: Single layer images of the base materials used.

Surfaces were imaged using a scanning electron microscope (JEOL LV6060) and a lab-scale RS PRO USB digital microscope.

Electron microscopy.

The microstructure was observed with SEM (LV6060, JEOL, Tokyo, Japan). Before all the tests, samples were cut into 5 mm × 5 mm cubical pieces coated with gold particles using a Gold and Platinum Sputter Coater. Specimens were observed at different magnifications.

Viruses and cells.

Vero E6 cells were originally obtained from Prof. Kin-Chow Chang (University of Nottingham) and maintained in minimal essential medium (MEM; Sigma) supplemented with 10% foetal calf serum (FCS; Sigma), 1% Penicillin/Streptomycin (Sigma) and 2mM L-Glutamine (Sigma).

The GLA-1 infectious variant of SARS-CoV-2 is an infectious clone developed from the original isolate of SARS-CoV-2 (17) and was obtained from the Centre for AIDS Reagents, NIBSC, UK. SARS-CoV-2 stocks were grown and quantified as described previously for other human coronaviruses (18).

Schmallenberg virus was obtained from the Frederich Loeffler Institute Germany was grown and quantified in Vero E6 cells as previously described (19) in Dulbecco's modified eagles Media (DMEM; Sigma) supplemented with 10% FCS (Sigma), and 2mM L-Glutamine (Sigma).

Testing of antiviral activity of materials.

In these assays, a 1-log drop in virus titre was considered an antiviral material. This is consistent with what would be required by the International Organisation for Standardisation (ISO) standards for antiviral activity of materials for both textile materials (ISO 21702) and non-porous surfaces (ISO 18184), though we did not attempt to meet all of those standards during these studies.

A non-sterile piece of each material (Supplementary material 1) was excised from the main source material and placed into the well of a 96-well plate (Thermo Scientific) for SARS-CoV-2 or a 12 well plate (Thermo Scientific) for SBV. The material was cuto to a size that comfortably fit flat ontoo the surface of the plate, but not so small that a 10μL drop would not fit.

For Schmallenberg virus, 2.8 x10^5 $TCID_{50}$ of infectious SBV was spotted onto the surface of the materials in 10μL of fresh MEM supplemented with FCS (Sigma) and 2mM L-Glutamine (Sigma) for 15 minutes with the material. The material was then washed in 1ml PBS to recover virus and then a 1:2 dilution of the wash was applied to Vero cells in 96 well plates for the $TCID_{50}$ assay (19).

For SARS-CoV-2, 7.3 x10^3 *$TCID_{50}$* of infectious SARS-CoV-2 were spotted onto the surface in 10μL of fresh Vero E6 growth media and left for 10, 30 or 60 minutes. After this, SARS-CoV-2 was washed from the surface in 200μL of fresh MEM supplemented with FCS (Sigma), 2mM L-Glutamine (Sigma) and 1% Penicillin/Streptomycin (Sigma). The amount of SARS-CoV-2 in the media was then quantified by *$TCID_{50}$ assay (18).* For RNA experiments, the same material samples with SARS-CoV-2, were submerged in 500μL of TRIzol reagent (Ambion). The entire

sample was recovered and the RNA was extracted using the DIrectZol kit (Zymo Research) according to the manufacturers' instructions. SARS-CoV-2 RNA was quantified using primers targeted to the RNA-dependent-RNA polymerase (20). SARS-CoV-2 RNA was assessed using the QuantiNova® SYBR ® Green RT-PCR kit (Qiagen) and a FAST 7500 Real-Time PCR System (Applied Biosystems), both according to the manufacturers' instructions. C_t values for 'positive' samples were in the range of 25-35 (data not shown). Negative samples often gave no C_t value, these were assigned the number 40 (the maximum possible cycle number) for calculation purposes (data not shown). Relative expression was determined using the deltaC_t method, compared to the no material control (i.e. SARS-CoV-2 in the well of a 96-well plate).

Quantification of Schmallenberg and SARS-CoV-2 viruses by $TCID_{50}$ assay.

Schmallenberg virus $TCID_{50}$ was performed as previously described (19). Schmallenberg virus in suspension in cell culture media was used as a positive control and the cell culture medium with no virus as a negative control. A % reduction in virus titre compared to the control and a log reduction in virus concentration was calculated.

SARS-CoV-2 $TCID_{50}$ assay was performed using the same method as previously described for other coronaviruses (18). Relative recovered SARS-CoV-2 was calculated by comparison to a no material control.

Statistics.

All data were analysed using a one-way ANOVA and Dunnetts' multi-comparison test using Prism (GraphPad). Statistical significance was assumed where $p<0.05$.

Results and Discussion

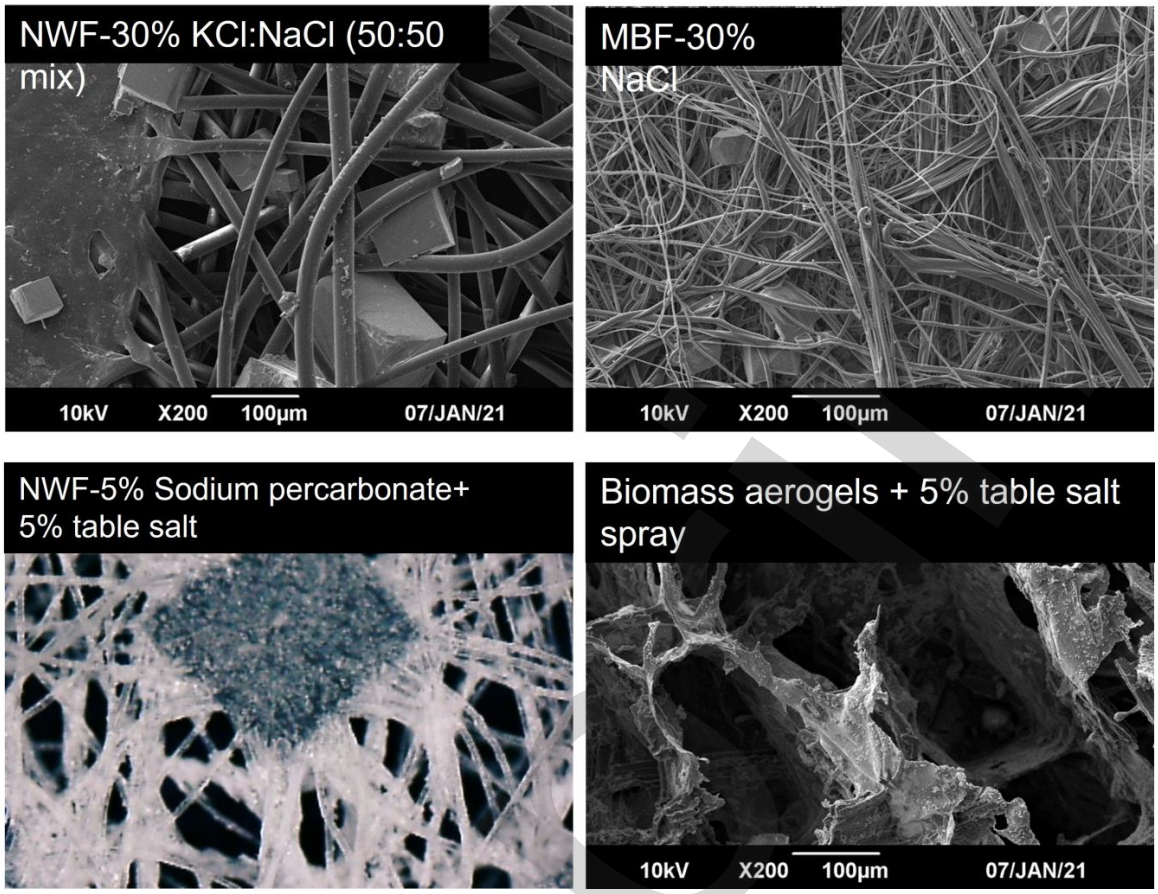

Figure 2: Salt deposit onto various surfaces.

Successful deposit of salt onto surfaces was confirmed through the observation of small salt particles in the materials using SEM (Figure 2) and digital microscopy (data not shown).

Preliminary screening of materials for antiviral activity against Schmallenberg virus.

Initially all materials listed in Supplementary Material 1 were tested for antiviral activity against Schmallenberg virus. These results yielded 16 materials that showed antiviral activity against Schmallenberg virus (materials highlighted in grey in Supplementary Material 1). SBV was chosen for these experiments because it easier to handle and readily replicates in Vero E6 cells, the cells used for the SARS-CoV-2 work and, therefore, was a more readily reproducible surrogate.

Schmallenberg virus was used as alternative commonly-used surrogate viruses (such as feline coronavirus or low pathogenicity avian influenza) do not always grow readily in the same cell lines as SARS-CoV-2. Additionally, tools for other hCoVs (apart from SARS-CoV-1 and MERS-CoV, which have the same biosafety concerns as SARS-CoV-2) are often less well developed and the viruses do not always cause the robust cell death that allows for a rapid screening process.

Antiviral activity of materials against SARS-CoV-2.

The antiviral materials from the Schmallenberg screen (Supplementary Material 1, highlighted in grey) and some additional materials (Supplementary Material 2) were tested for antiviral activity against SARS-CoV-2. Virus was added to the surface and left in contact for 10 minutes and, then, recovered virus quantified by $TCID_{50}$ assay. In line with ISO standards 21702 and 18184, A material was considered to be a 'hit' if the virus titre was lowered by at least 1-log from the control (SARS-CoV-2 on the surface of the 96-well plate) run in parallel. All 'hit's from the Schamallenberg virus screen also showed antiviral activity against SARS-CoV-2 and, all together these results yielded 16 materials that showed antiviral activity against SARS-CoV-2 virus (materials highlighted in grey in Supplementary Material 1 and Supplementary Material 2).

To further determine the anti-SARS-CoV-2 activity of each material, the data from the screen were repeated and antiviral activity was also tested over a longer contact time. For ease of labelling, each hit material and one control (a material that had no effect on virus titre) was assigned a number, as follows:

Table 1: Numbers identifying each material in figures

Assigned number	Material	
1	Face mask coated with 30% NaCl:KCl (50:50 mix)	Middle layer (melt-blown fabric)
2	Face mask coated with 30% NaCl	Middle layer (melt-blown fabric)
3	Non-woven fabric coated with sodium percarbonate at shown %	5% + 5% table salt
4		5% + 3% table salt
5	Bioaerogel with 20% salt and 2% TiO_2	
6	Table salt spray on non-woven fabric at shown %	5
7		10
8		15
9		20
10	30% KCl:NaCl (50:50 mix) on non-woven fabric	
11	Bioaerogel-KIG2S4W52 at shown %	50 + 5% salt spray
12		70 + 5% salt spray
13		90 + 5% salt spray
14		50 + 20% salt spray
15		70 + 20% salt spray
16		90 + 20% salt spray
17 (control)	Uncoated face mask material	Middle layer (melt-blown fabric)

The 17 materials in table 1 were tested for anti-SARS-CoV-2 activity at 10, 30 and 60 minutes post-SARS-CoV-2 addition (Figure 3).

At 10 minutes post-SARS-CoV-2 addition (Figure 3A), the results were variable, with some materials showing more variation than others. However, materials 6, 9, 10 and 11 showed consistent recovered titres of greater than 1-log reduction compared to the no coating control (Figure 3A, red line shows 1-log drop). None of the coatings at this time point consistently achieved no virus recovery (Figure 3A, blue line shows detection limit). When analysed statistically all samples, except sample 5, showed statistically significantly different titres compared to the control (one way ANOVA and Dunnetts' multi-comparison test; $p<0.05$), suggesting that the coatings did significantly affect SARS-CoV-2 stability.

By 30 minutes post-SARS-CoV-2 addition (Figure 3B), materials 1, 7, 8, 9, 10, 11, 12, 13 14, 15 and 16 were able to consistently lower the virus tire by at least 1-log compared to the no coating control. There was no SARS-CoV-2 recovered from materials 7, 11, 12, 13 or 15 (Figure 3C). Only material 5, did not consistently show at least a 1-log drop in recovered SARS-CoV-2 titre

compared to the no coating control (Figure 3B). When analysed statistically all samples, except samples 2 and 5, showed statistically significantly different titres compared to the control surface (one way ANOVA and Dunnetts' multi-comparison test; $p<0.05$), suggesting that the coatings did significantly affect SARS-CoV-2 stability.

By 60 minutes post-SARS-CoV-2 addition (Figure 3C), all materials, including material 5, were able to consistently lower the virus tire by at least 1-log compared to the no material control. There was no SARS-CoV-2 recovered from any material 8, 11, 12, 13 or 14 samples (Figure 3C) and there was no recovered SARS-CoV-2 in 2/3 samples with materials 7 and 10 (Figure 3C). Material 6, did not consistently show at least a 1-log drop in recovered SARS-CoV-2 titre compared to the no material control (Figure 3C), but did show a drop in titre compared to control of approximately 67%. When analysed statistically none of the samples were significantly different from the control surface (one way ANOVA and Dunnetts' multi-comparison test; $p>0.05$), suggesting that the coatings did not significantly affect virus stability. However the uncoated surface also had a significant drop in titre compared to the no material control, so exposure to any surface at this time point reduced SARS-CoV-2 stability.

Overall, all of the tested materials were able to significantly drop SARS-CoV-2 titre over time, with some showing complete destruction of SARS-CoV-2 infectivity.

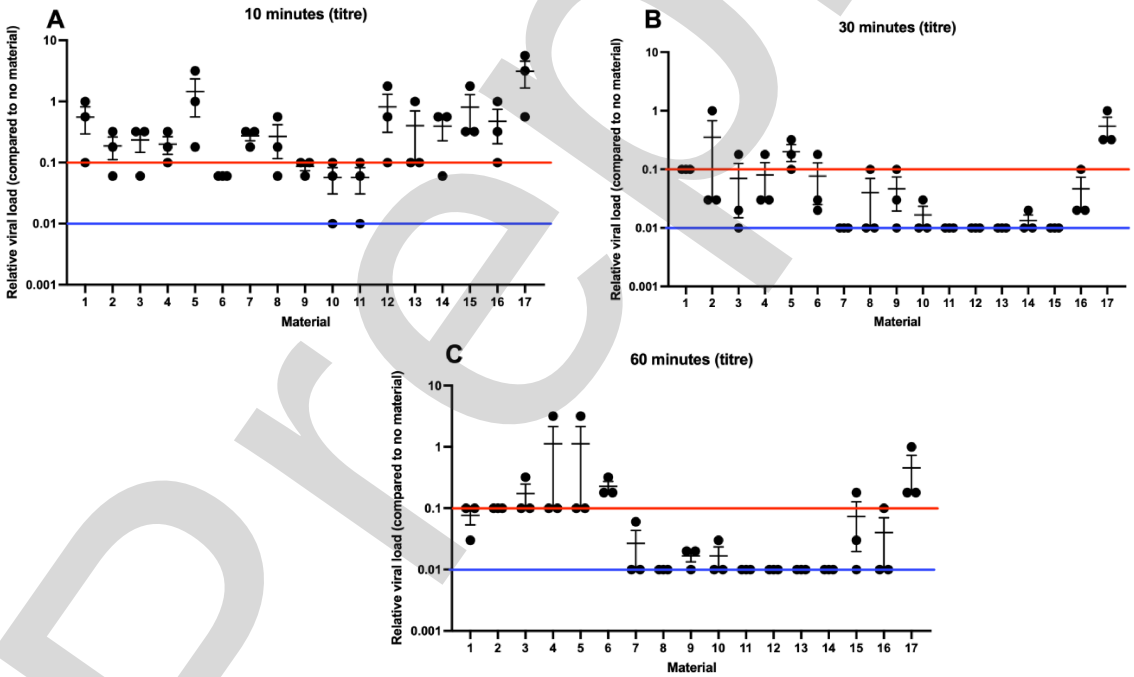

Figure 3: Recovered SARS-CoV-2 after 10 minutes (A), 30 minutes (B) or 1 hour (C) exposure to materials. Numbers correspond to materials stated in table 1. Red line indicates a 1-log drop compared to control. Blue line indicates limit of detection. Individual data points and the mean ± SEM are shown.

Detection of SARS-CoV-2 RNA after contact with surfaces.

Because the only viral RNA that should be present in the virus preparation should be genomic RNA, this is a measure of physical virus presence in a sample. However, the virus may itself be either infectious or inactivated. To determine if there is evidence of SARS-CoV-2 RNA degradation, RT-PCR was performed on material samples that had had contact with SARS-CoV-2 for 10, (Figure 4A), 30 (Figure 4B), or 60 (Figure 4C) minutes. These data were much more variable than the titre data, in that most samples did not show consistent reduction of SARS-CoV-2 RNA within or across timepoints (Figure 4A, B and C; red line indicates a 1-log drop). Materials 3, 6, 8, 9, 10, 13, 14 and 16 consistently showed a 1-log or greater drop in SARS-CoV-2 RNA at at least one timepoint post-contact, but most of these did not show a drop across all timepoints. The other materials never consistently showed a greater than 1-log drop in SARS-CoV-2 RNA at any timepoint post-contact (Figure 4A, B and C; red line indicates a 1-log drop). When compared statistically, none of the samples showed any significantly different RNA compared to the control surface (one way ANOVA and Dunnetts' multi-comparison test; $p<0.05$ in all cases).

Although these data are highly variable, perhaps due to differences in the absorbance of the surfaces, we can at least conclude that SARS-CoV-2 RNA can still be detected in association with most of the surfaces at the various time points. Taken together with the $TCID_{50}$ data, the data suggest this is not infectious SARS-CoV-2 suggests that fragments of SARS-CoV-2 RNA can remain on the surfaces for longer periods of time.

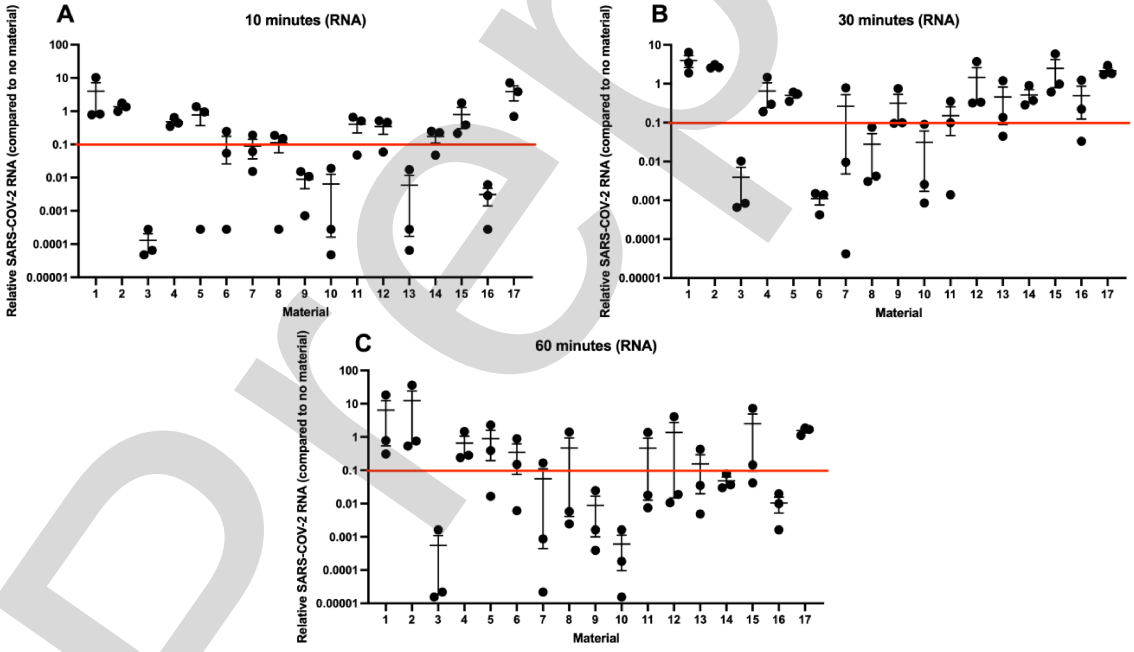

Figure 4: Recovered SARS-CoV-2 RNA after 10 minutes (A), 30 minutes (B) or 1 hour (C) exposure to materials. Numbers correspond to materials stated in table 1. Red line indicates a 1-log drop compared to control. Individual data points and the mean ± SEM are shown.

Conclusions

Emerging new virus variants and waning immunity due to infection or vaccination mean that effective non-pharmaceutical intervention remains a critical part of protecting the public against ongoing and future outbreaks of SARS-CoV-2 and other respiratory viruses. In this study we identify a number of non-expensive and scalable salt formulations that, in line with the ISO standard a 1-log drop in titre, have anti-viral activity against SARS-CoV-2 when used as a coating on common facemask fabrics and could, therefore, to control spread of SARS-CoV-2.

Our observations are in line with previous studies that have shown salt coating of surfaces can be an effective tool to prevent the spread of respiratory pathogens (10) showing anti-bacterial (11) and antiviral (12, 13) properties. In this study we have shown combination of Bioaerogel and salt spray are particularly effective in inactivating SARS-CoV-2 by at least 1-log after exposure of only 30 minutes, with 5% salt spray showing this as early as 10 minutes post-exposure. Given that the presence of water has been specifically implicated to help with SARS-CoV-2 stability (21), it is reasonable to assume that the anti-viral coatings is, at least partly, due to their potent desiccant properties. Interestingly, our data also indicate that the function of the anti-viral coatings is not influenced by the nature of the substrate they are applied on. This means these salt coatings could be potentially applied on different existing materials and their use is not restricted to specific materials.

The detection of viral RNA on most of the surfaces suggest that that the surfaces do not cause the complete destruction of all viral components. Some of the materials, however, do appear to cause the complete degradation of SARS-CoV-2, such that not even fragments of SARS-CoV-2 RNA can be detected.

We acknowledge that these are *in vitro* studies that may not be applicable to the real-world, but these data are an important first step in the process. The use of the biomass aerogels, in particular, will be a key area of future study. We previously reported on the biophysical and filtration properties of similar biomass aerogels to those used in this study (15, 16). One concern, for example, would be the breathability of novel facemask materials, such as the biomass aerogel (11). Although we did not test this as part of this study, previous work suggests that a similar biomass aerogel had a low filtration resistance (15). Additionally, although the pore size of biomass aerogels is large (16), we have previously showed that the overlapping network of pores creates a network that should block most viruses (16).

In short, in this study we have shown that spray costing of different type of fabric used in making facemasks provides potent anti-viral properties against SARS-CoV2 and can be used a fast and non-expensive method for developing more effective personal protective equipment against respiratory viruses. Further work will determine the exact mechanism of action of these coatings and determine the utility and efficacy of the anti-viral masks in real world settings.

Data summary

No new data, tools, software or code have been deposited, except as shown in the paper itself. Data all figures has been deposited at microbiology.figshare.com (22).

Author contributions

CMC: conceptualisation, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualisation, funding acquisition. **BW:** methodology, validation, formal analysis, investigation, data curation. **YW:** methodology, validation, investigation, resources, writing – review and editing, visualisation. **ETB:** methodology, validation, resources, writing – review and editing. **ZC:** methodology, validation, resources. **JR:** methodology, validation, resources. **SR:** conceptualisation, writing – review and editing, supervision, project administration, funding acquisition. **ST:** resources, writing – original draft, writing – review and editing, supervision. **AG:** conceptualisation, writing – review and editing, supervision, project administration, funding acquisition.

Conflict of interest statement

There are no conflicts of interest.

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Data is deposited at microbiology.figshare.com

Reviewer 2.

We thank this reviewer for re-reviewing the paper and note that they now have no issues with this proceeding to publication.

Reviewer 3.

Several times in the manuscript reference is made to a one-log drop in viral activity being in line with ISO standards. A one-log drop is equivalent to 90% reduction in activity, this is a minimally accepted value and most products that claim to be anti-bacterial or virucidal are expected to achieve a 3 log drop e.g "kills 99.9% of all know germs". I have checked both of the ISO standards cited (ISO 21702 and ISO 18184) and I cannot see any reference to a one-log drop in these? Please can the source of this claim be clarified?
We believe the wording in that section clarifies that our work is based on these standards, but we are not claiming we fully meet them (indeed we state this). We have also toned down some more categorical references to the standards in later sections, which were added in response to a comment in the first round.

It appears that different sizes of the test materials were used? 12cm x 10cm and 15cm2 - and different methods for coating the materials - soaking and spraying - how can these be compared?
In this paper, we are assessing the antiviral capacity of the materials and, in fact, using only a small piece of each material to do so. Although it is, of course, important to record exactly how each material was made – these have no effect on the downstream antiviral assay. Further publications on the materials themselves will, of course, take this into account as that will be a critical aspect for creation of the correct material.

L.130 - Vero E6 growth media should be changed to MEM + 10% FCS (if that is what was used)
Corrected. And also the same issue a few sentences later.

L.131 isn't clear - why were the virus stocks used? Do the authors mean the washings from the material? "applied to vero cells in 96 well plates with TCID50 assay" doesn't make sense? Maybe for TCID50 assay?
Corrected – the material was washed to recover the virus for the $TCID_{50}$.

L.136 what size of material was used for the RT-PCR experiments? What volume was recovered? What volume was tested in the assay?
Corrected with additional details.

L.142-143 - what is the "no material control"? just virus? Is there a material without salt coating control? As mentioned above this may in itself absorb virus resulting in a reduced titre for recovered washings?

Detail has been clarified. As stated later in the paper, we used uncoated surfaces thoughout (sample 17) as a control for the material itself.

L. 168-170 - I am unsure of the importance of Schmallenberg virus replication in the same cell line as SARS-CoV-2? Is this just convenience to avoid having to propogate 2 cell lines? There is no direct comparison being made between the 2 viruses and indeed substantially different titres and exposure times have been used so why is this relevant?
We fully acknowledge that Schmallenberg virus is not a perfect surrogate for SARS-CoV-2 and would not claim as such. The language in the manuscript highlights some advantages of this virus over other alternatives – but we completed all of the work with live SARS-CoV-2 after the preliminary screen using a virus that tried to capture as many features of the high containment experiments that followed (an enveloped virus that replicates in Vero E6 cells and is easy to handle and quantify).

Table 1 - the only test material control used appears to be uncoated face mask? This cannot act as a control for the other materials and is therefore only relevant for numbers 1 and 2
This is correct, we do not have all of the uncoated materials. However, we believe this a valid control as an uncoated material that is currently in use. The key comparisons in this paper are to no material. We are not making any claim about the underlying materials.

L.221-223 - Agreed - the only "fair" way to conduct this test is to use uncoated material of each type as the control and compare virus recovered from that material with virus recovered from the coated material. Most materials will cause a reduction in viral titre over time.
We respectfully disagree that uncoated materials are required for all of the comparisons. We used uncoated face mask material as the current standard to compare to and showed very little reduction from the, in effect, virus in liquid.

What Ct values were obtained for the RT-PCR? only the relative ratios compared to no-material control are presented.
We fully appreciate that the delta Ct can be used to 'hide' poor Ct values. However, all Ct values were in a valid range for comparison (around 20 for a positive sample and none (corrected to 40 for comparison purposes) for a true negative). A note has been added to the materials and methods to reflect this.

L.234-235 RT-PCR is not more sensitive that TCID50 - they measure different things. RT-PCR detects viral RNA whether from a live or inactivated virus particle. TCID50 is a measure of viral infectivity. This should be amended.
This has been amended to remove the reference to sensitivity.

L.235 - 252 - Again, in the absence of test material controls it is difficult to draw conclusions about the RT-PCR data. The results are highly variable and show no correlation with time of exposure. The most likely explanation is variability in the absorbance of each test material and in volume recovered after exposure. Was any

external control (e.g. MS2 RNA) used to ensure the assay worked consistently? Could some comment on this be made
We appreciate that the RNA data are variable and have added a comment to that effect in the relevant results section. We appreciate we did not include the control, however the more interesting data are probably those where there is still viral RNA present, despite a drop in titre, rather than any 'false negatives' caused by material absorbance.

I do not think it is justified to conclude that either the data (in conjunction with TCID50) show it is non-infectious virus (L.247-250) or that some of the materials....cause complete degradation....(L.251-252) this is just speculation - maybe include in the discussion rather than results.
These sentences have been moved to the discussion.

L.28 Insert "usually": The seasonal hCoVs, such as hCoV-299E and hCoV-OC43, usually cause mild.....
Added.

L.31 as far as I am aware the first SARS virus is just called "SARS" not "SARS CoV-1"?
I have done a search and there is now a growing acceptance, it appears, that the original SARS should be referred to as SARS-CoV-1. Of course, this can be quite confusing because all papers before 2019 will simply have referred to it as SARS-CoV (there was no -2). I have added a short 'previously known as' to the relevant sentence.

L.34-35 - It may be true that the reason for the geographical restriction of MERS is due to the zoonotic host - although the original source of MERS is still debated - The virus is now endemic in camels in the Middle East and transmission to humans occurs from close contact with the camels with poor human-human spread limiting its geographical spread (not highly restricting it though, it has been found in humans in 27 different countries). However, this is not true for SARS. SARS did spread human-human so the geographical habitat of civet cats is not a factor. It was contained by quarantining humans. Why it hasn't re-emerged isn't understood. But if the argument used here were true it would keep re-emerging wherever humans were in contact with civet cats.
Suggest rewriting this paragraph to reflect the above and remove "highly" restricted
The paragraph has been edited to remove civet cats from the geographical distribution comment. "Highly" has also been removed.

L.35 "civet" not "civit"
Corrected (and also moved).

L.41 "coronaviruses emerging into the human population hCoVs, causing...." This line doesn't make sense - change to ..."coronaviruses emerging into the human population as human coronaviruses (hCoVs), causing...."
Corrected and apologies for the typographical error there that was missed during drafting.

L.41-42 - update to the latest COVID figures from the WHO dashboard?
Have updated the figures and the date.

L.45 - I disagree with the statement that there was "relatively little interest in hCoVs as human pathogens" - they are well recognised as causing significant morbidity and even mortality in some patient groups and the economic cost of the "common cold" is huge. It would be better to say something like:
Human coronaviruses have been recognised as a significant cause of the "common cold" since the 1960's when they were first identified, but despite the emergence of SARS in 2002-3 had not been seen as having major pandemic potential
We have made the change (with minor changes to the wording) as suggested.

L.61 - (even under relaxed regulatory requirements that promote rapid development) - this is an incorrect and potentially dangerous statement. The regulatory requirements were not relaxed. They were speeded up - at financial risk to the manufacturers who began vaccine production before results of phase III clinical trials had been obtained. This was the main reason for the accelerated production of the vaccines, there was no relaxation in terms of safety evaluation of the vaccines. This statement must be removed or significantly amended.
This statement has been removed, it was intended to imply that safety had been compromised, but appreciate it could have been read that way. Apologies.

How practical would a salt coated surface or textile be? Wouldn't the salt be easily removed via normal day-day activities? Apart from a brief citation to reference 9 little discussion of the many existing anti-viral coatings for face masks (e.g. copper) that emerged early in the pandemic is made. There are numerous examples in the literature and on commercial websites
We acknowledge that practicalities may mean this is not an appropriate mechanism going forward. This is acknowledged in the final sentence of the paper. We have used reference 9 as a review of the topic that cites the other studies in this area, rather than citing them all individually.