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SHORT COMMUNICATION
Coleman *et al.*, *Access Microbiology* 2023;5:000492.v5
DOI 10.1099/acmi.0.000492.v5

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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 coatings 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.

DATA SUMMARY

Supporting data including Supplementary Material 1 and 2 for Antiviral activity of salt-coated materials against SARS-CoV-2 are deposited at https://doi.org/10.6084/m9.figshare.22587607.v1 [1].

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, [2]). 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 [3]. MERS-CoV continues to cause human infections, but is primarily a camel virus [4] and cannot spread between humans easily under normal conditions [5]. 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).

Received 09 September 2022; Accepted 04 September 2023; Published 27 September 2023
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Keywords: coronaviruses; materials; respiratory medicine; virus stability.
Abbreviations: ANOVA, analysis of variance; hCoV, human coronavirus; ISO, International Organisation for Standardization; MERS-CoV, Middle East respiratory syndrome coronavirus; NWF, non-woven fabric; RNA, ribonucleic acid; RT-PCR, reverse transcriptase polymerase chain reaction; SARS-CoV, severe acute respiratory syndrome coronavirus; SBV, Schmallenberg virus; SEM, scanning electron microscope; TCID, tissue culture infectious dose.

Human coronaviruses have been recognized 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 antiviral 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 [6, 7]. The properties of each mask, including material, fit to the face and filtration capacity can have a big impact on their efficacy [8, 9]. 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 a 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 [10]). But, salt coating of various materials has been proposed as an effective tool to prevent the spread of respiratory pathogens [11] and they have previously been shown to be antibacterial against a range of important human pathogens [12]. Additionally, salt coating of surfaces can be antiviral, both *in vitro* and, interestingly, *in vivo* [13, 14]. Specifically, coating surfaces prevented the spread of influenza viruses by inactivating viruses that passed through the coated filter [14] and reduced the stability of a pig coronavirus, transmissible gastroenteritis virus [13].

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

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 (Fig. 1) were developed and provided from the Hubei University of Technology [15]. Surgical mask was provided by KMD Company.

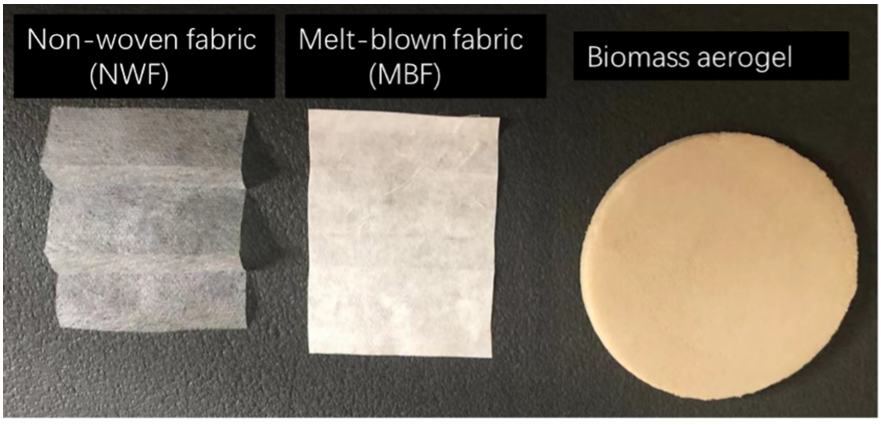

Fig. 1. Single-layer images of the base materials used.

Non-sterile 12 cm by 10 cm pieces of non-woven fabrics and melt-blown fabrics (Fig. 1) were soaked in the 200 ml of each salt solution and then dried in a 50 °C drying oven. A small humidifier was used to spray 5 ml salt water onto the surface of non-sterile 15 cm^2 pieces of biomass aerogels and then dried with a hairdryer. For the facemask (KMD Company), 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 [16, 17].

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 Professor 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 2 mM 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 [18] 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 [19].

Schmallenberg virus was obtained from the Frederich Loeffler Institute Germany was grown and quantified in Vero E6 cells as previously described [20] in Dulbecco's Modified Eagle Media (DMEM; Sigma) supplemented with 10% FCS (Sigma), and 2 mM 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 cut to a size that comfortably fit flat onto the surface of the plate, but not so small that a 10 µl drop would not fit.

For Schmallenberg virus, 2.8×10^5 $TCID_{50}$ of infectious SBV was spotted onto the surface of the materials in 10 µl of fresh MEM supplemented with FCS (Sigma) and 2 mM L-glutamine (Sigma) for 15 min with the material. The material was then washed in 1 ml 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 [20].

For SARS-CoV-2, 7.3×10^3 $TCID_{50}$ of infectious SARS-CoV-2 were spotted onto the surface in 10 µl of fresh Vero E6 growth media and left for 10, 30 or 60 min. After this, SARS-CoV-2 was washed from the surface in 200 µl of fresh MEM supplemented with FCS (Sigma), 2 mM L-glutamine (Sigma) and 1% penicillin/streptomycin (Sigma). The amount of SARS-CoV-2 in the media was then quantified by $TCID_{50}$ assay [19]. For RNA experiments, the same material samples with SARS-CoV-2, were submerged in 500 µl 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 [21]. 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 [20]. 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 [19]. 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

Successful deposit of salt onto surfaces was confirmed through the observation of small salt particles in the materials using SEM (Fig. 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 is 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.

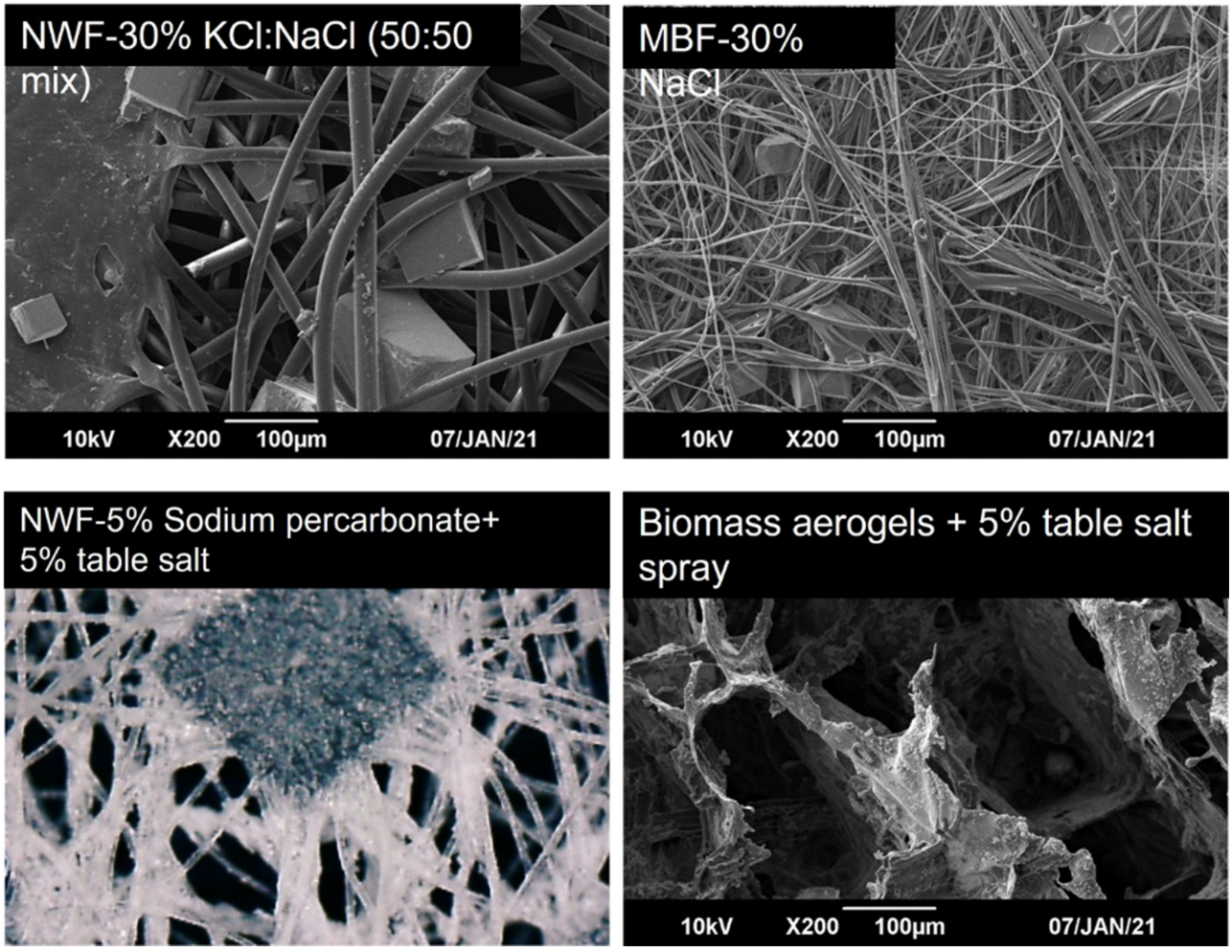

Fig. 2. Salt deposit onto various surfaces.

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 min and, then, recovered virus quantified by $TCID_{50}$ assay. In line with ISO standards 21 702 and 18 184, 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 1–17. The 17 materials in Table 1 were tested for anti-SARS-CoV-2 activity at 10, 30 and 60 min post-SARS-CoV-2 addition (Fig. 3).

At 10 min post-SARS-CoV-2 addition (Fig. 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 (Fig. 3a, red line shows 1-log drop). None of the coatings at this time point consistently achieved no virus recovery (Fig. 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 min post-SARS-CoV-2 addition (Fig. 3b), materials 1, 7, 8, 9, 10, 11, 12, 13 14, 15 and 16 were able to consistently lower the virus titre 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 (Fig. 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 (Fig. 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.

Table 1. Numbers identifying each material in figures

Assigned no.	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)

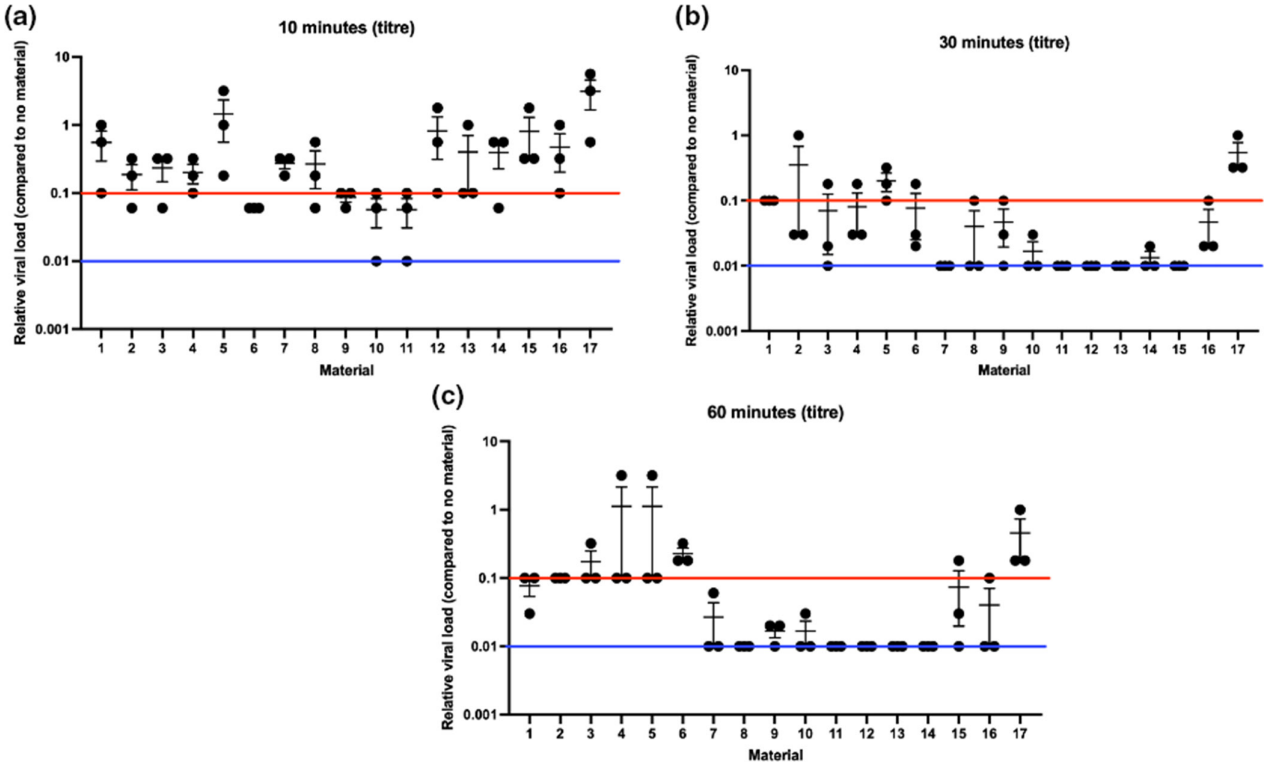

Fig. 3. Recovered SARS-CoV-2 after 10 min (a), 30 min (b) or 1 h (c) exposure to materials. Numbers correspond to materials stated in Table 1. Red line indicates a 1-log drop compared to control. Blue line indicates limit of detection. Individual data points and the mean±SEM are shown.

By 60 min post-SARS-CoV-2 addition (Fig. 3c), all materials, including material 5, were able to consistently lower the virus titre 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 (Fig. 3c) and there was no recovered SARS-CoV-2 in 2/3 samples with materials 7 and 10 (Fig. 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 (Fig. 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.

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, (Fig. 4a), 30 (Fig. 4b) or 60 (Fig. 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 (Fig. 4a–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 (Fig. 4a–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.

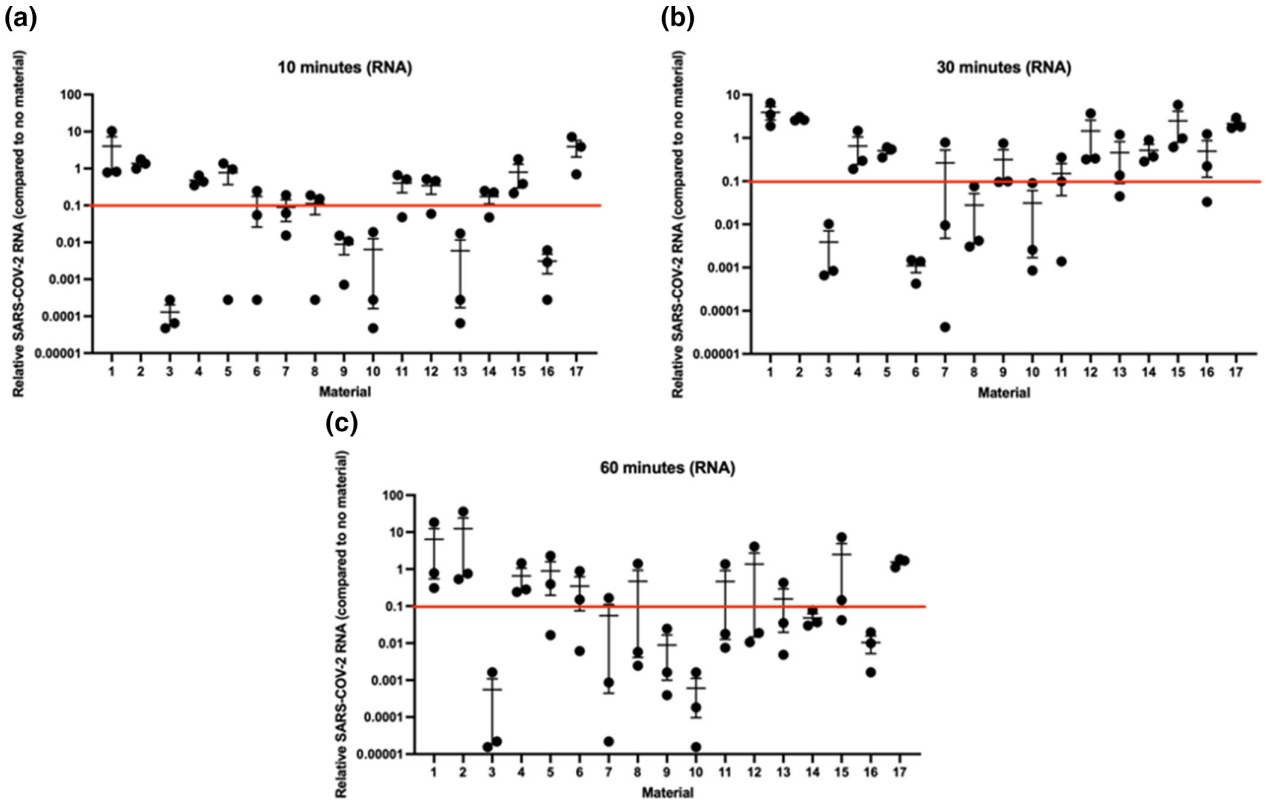

Fig. 4. Recovered SARS-CoV-2 RNA after 10 min (a), 30 min (b) or 1 h (c) exposure to materials. Numbers correspond to materials stated in Table 1. Red line indicates a 1-log drop compared to control. Individual data points and the mean±SEM are shown.

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 antiviral activity against SARS-CoV-2 when used as a coating on common facemask fabrics and could, therefore, 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 [11] showing antibacterial [12] and antiviral [13, 14] properties. In this study we have shown a combination of Bioaerogel and salt spray are particularly effective in inactivating SARS-CoV-2 by at least 1-log after exposure of only 30 min, with 5% salt spray showing this as early as 10 min post-exposure. Given that the presence of water has been specifically implicated to help with SARS-CoV-2 stability [22], it is reasonable to assume that the antiviral coatings is, at least partly, due to their potent desiccant properties. Interestingly, our data also indicate that the function of the antiviral 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 [16, 17]. One concern, for example, would be the breathability of novel facemask materials, such as the biomass aerogel [12]. Although we did not test this as part of this study, previous work suggests that a similar biomass aerogel had a low filtration resistance [16]. Additionally, although the pore size of biomass aerogels is large [17], we have previously showed that the overlapping network of pores creates a network that should block most viruses [17].

In short, in this study we have shown that spray coating of different types of fabric used in making facemasks provides potent antiviral properties against SARS-CoV2 and can be used as 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 antiviral masks in real-world settings.

Funding information
This project was funded through an Innovate UK rapid response grant awarded to SR, CMC and AG

Author contributions
C.M.C.: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualization, funding acquisition. B.W.: methodology, validation, formal analysis, investigation, data curation. Y.W.: methodology, validation, investigation, resources, writing – review and editing, visualization. E.T.B.: methodology, validation, resources, writing – review and editing. Z.C.: methodology, validation, resources. J.R.: methodology, validation, resources. S.R.: conceptualization, writing – review and editing, supervision, project administration, funding acquisition. S.T.: resources, writing – original draft, writing – review and editing, supervision. A.G.: conceptualization, writing – review and editing, supervision, project administration, funding acquisition.

Conflicts of interest
The author(s) declare that there are no conflicts of interest.

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Peer review history

VERSION 4

Editor recommendation and comments

https://doi.org/10.1099/acmi.0.000492.v4.1

James Redfern; Manchester Metropolitan University, UNITED KINGDOM

Date report received: 04 September 2023
Recommendation: Accept

Comments: Following your recent round of revisions, I am happy you have addressed all concerns from the reviewers, and we are happy to accept this manuscript for publication.

Author response to reviewers to Version 3

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.

VERSION 3

Editor recommendation and comments

https://doi.org/10.1099/acmi.0.000492.v3.3

James Redfern; Manchester Metropolitan University, UNITED KINGDOM

Date report received: 08 August 2023
Recommendation: Major Revision

Comments: The reviewers have highlighted major concerns with the work presented. Please ensure that you address their comments.

Reviewer 2 recommendation and comments

https://doi.org/10.1099/acmi.0.000492.v3.1

Pamela Vallely; University of Manchester School of Biological Science: The University of Manchester Faculty of Biology Medicine and Health, Evolution, Infection and Genomic Medicine, UNITED KINGDOM
https://orcid.org/0000-0002-7712-9131

Date report received: 08 August 2023
Recommendation: Major Revision

Comments: 1. Methodological rigour, reproducibility and availability of underlying data Whilst I appreciate that the authors have not attempted to conduct these experiments in line with ISO standard methods there are some fundamental principles that need to be adhered to in order to be able to validate the results. As I understand it, the controls used in the experiments (against which the reduction in viral titre after exposure to the materials is measured) is virus suspension that has not been exposed to the material. As all porous material will absorb virus instantly, the ISO standard for textiles requires the control to be virus recovered from the textile immediately post-exposure as this gives a much more realistic comparison to measure the antiviral effect of the textile. This has not been done here and so it should be acknowledged that the base line viral titre used is likely to be higher than it should be. In addition the only non-coated material used is face mask which is not a valid control for the other materials. 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? Materials - 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? L.130 - Vero E6 growth media should be changed to MEM + 10% FCS (if that is what was used) 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? L.136 what size of material was used for the RT-PCR experiments? What volume was recovered? What volume was tested in the assay? 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? 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? 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 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. 2. Presentation of results Generally the results are clearly presented. The below suggestions would help: What Ct values were obtained for the RT-PCR? only the relative ratios compared to no-material control are presented. 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. 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? 3. How the style and organization of the paper communicates and represents key findings The methods are difficult to follow in parts and would be hard to replicate. 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. 4. Literature analysis or discussion L.28 Insert "usually": The seasonal hCoVs, such as hCoV-299E and hCoV-OC43, usually cause mild….. L.31 as far as I am aware the first SARS virus is just called "SARS" not "SARS CoV-1"? 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 L.35 "civet" not "civit" 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…." L.41-42 - update to the latest COVID figures from the WHO dashboard? 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 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. 5. Any other relevant comments 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.

Please rate the manuscript for methodological rigour
Poor

Please rate the quality of the presentation and structure of the manuscript
Satisfactory

To what extent are the conclusions supported by the data?
Partially support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?
No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
Yes

Reviewer 1 recommendation and comments

https://doi.org/10.1099/acmi.0.000492.v3.2

Anonymous.

Date report received: 30 May 2023
Recommendation: Accept

Comments: The previous reviewer comments have all been addressed and I am happy to recommend this manuscript for acceptance.

Please rate the manuscript for methodological rigour
Very good

Please rate the quality of the presentation and structure of the manuscript
Very good

To what extent are the conclusions supported by the data?
Strongly support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?
No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
Yes

Author response to reviewers to Version 2

Data is deposited at microbiology.figshare.com

VERSION 2

Editor recommendation and comments

https://doi.org/10.1099/acmi.0.000492.v2.1

James Redfern; Manchester Metropolitan University, UNITED KINGDOM

Date report received: 11 April 2023
Recommendation: Major Revision

Comments: Please deposit the data underlying the work in the Society's data repository Figshare account here: https://microbiology.figshare.com/submit. Please also cite this data in the Data Summary of the main manuscript and list it as a unique reference in the References section. When you resubmit your article, the Editorial staff will post this data publicly on Figshare and add the DOI to the Data Summary section where you have cited it. This data will be viewable on the Figshare website with a link to the preprint and vice versa, allowing for greater discovery of your work, and the unique DOI of the data means it can be cited independently. Before we send your manuscript back to reviewers following your revision, please ensure all data underpinning your work (for example, data that went into figures 3 and 4), are shared via FigShare (or other similar platform) and detailed in the Data Summary section.

Author response to reviewers to Version 1

Reviewer 1

Abstract section needs to be expanded upon so that the reader gets a sense for the direction the article will take and what the authors found. For example, was there a single coating that was more effective in all test materials? Did the material type have any impact on antiviral activity given similar coatings? What else did the authors conclude from the study? How does this information add to the existing body of knowledge?

Abstract has been rewritten.

Descriptions of fabric treatments in the Materials section needs to include more procedural detail so that the testing could be repeated by others, if desired. For example, what were the fabric swatch sizes, salt solution volumes, and drying oven temperature? Also, Appendix 1 needs to indicate if by "face mask" this means melt-blown fabric since nonwoven fabric is listed.

Details have now been added to the materials section.

The sample size is 12 cm*10 cm. The non-woven fabric and melt-blown fabric were soaked in 200 mL salt solution until fully wetted and then transferred into the drying oven at 50°C.

The inner and outer layer of the face mask is non-woven fabric and the middle layer is melt-blown fabric.

It would be better to have SEM photomicrographs seen in Figure 2 show the common treatment, that being 30% KCl:NaCl (50:50 mix). It is difficult to compare the relative distribution of salt coating from one material to the other when four different treatments are shown.

The figure has been revised as per concern.

The authors need to provide a rationale for testing the outer, middle, and inner layers of the "face mask" in Appendix 1 using the Schmallenberg virus. This process was not mentioned in the Materials section.

This process was described in the methods section under "Testing of antiviral activity of materials".

Also, it should be specified if non-woven and melt-blown fabric samples seen in Figure 1 are intact 3-layers or a single separated layer.

The inner and outer layer of the face mask is non-woven fabric and the middle layer is melt-blown fabric.

The pore sizes shown in Figure 2 appear to be much too large to ensure adequate contact of aerosolized virus particles with incorporated salt material. The Centers for Disease Control and Prevention (CDC) states that the average particle size of SARS-CoV-2 is around 0.1 micrometer (μm). Also, the minimum size of a respiratory particle that can contain SARS-CoV-2 is calculated to be approximately 9.3 μm.* Judging by the 100 μm size bar, the pore sizes in these materials, with the possible exception of MBF-30% NaCL, would be much too large for an appreciable numbers of virus particles to come into contact with the salt-coated material. Physical contact would be required to for virus inactivation via desiccation.

Additional discussion of this point has been added, based on the previous study of these materials (reference 16).

An additional practical concern would be the breathability (ease of breathing) using a mask coated with NWF-NACL&KCL 30%. This would be questionable given that the provided SEM photomicrograph is representative of several examined fields. This type of limitation should also be discussed since this same issue is mentioned in one of their provided references.**

Additional discussion of this point has been added, based on the previous study of these materials (reference 15).

The authors should supply the commercial source of face mask materials.

Detail has been added. The face mask was supplied by KMD COMPANY LIMITED.

Lines 25-29 - Suggest mentioning that the geographic restriction was most likely related to a limited geographical distribution of zoonotic sources for SARS-CoV-1 (civet cats) and MERS-CoV (dromedary camels).

Change made

Line 32 - Recommend changing "well in normal situations" to "easily under normal conditions".

Change made

Line 35 - The zoonotic source of SARS-CoV-2 has not been definitively established. Recommend sticking to statements indicating the relative ease by which this virus spreads from human-to-human.

Change made

Lines 39-40 - Suggest describing the typical affliction brought on by hCoVs, pre- SARS-CoV-2, that being a head cold that was not geographically restricted in its incidence. hCovs were deemed responsible for ~20% of common colds until the appearance of

Change made

Lines 52-53 - The authors should provide examples of attempts to incorporate antiviral materials (e.g., metallic nanoparticles) into facemasks and what types of masks (e.g., cloth-single vs cloth double layer) were used in these attempts.

Have added in a reference to a review that summarises a number of these.

Lines 84-85 - The authors should specify the amount of salt solution applied to the biomass aerogel material. The same goes for non-woven fabrics and melt-blown fabrics soaked in salt solutions. Additionally, how big were salt-treated fabric sizes used for testing?

Details have been added. For 15cm*15cm biomass aerogels, the 5 ml salt solution was used to spray, which is different from the non-woven fabric and melt-blown fabrics. The fabrics were soakedin 200 mL salt solution and then transferred into a drying oven at 50°C.

Line 105-107 - The authors should explain the reason 2 greatly different microplates were used for testing either SARS-CoV-2 or SBV. This implies a rather large difference in sample size used to test either virus.

Sample volumes added were the same (10ul) and virus amounts were determined by stock concentrations. The size of the plate is irrelevant.

Line 115 - Viruses are nonliving, so the word "active" should be used in lieu of "live".

Changed to "infectious".

Line 163 - Was a material control also included? A lowering of the virus titer should be compared to an untreated material control and not to a "no material" control. It cannot be reasonably assumed that the material by itself does not possess some antiviral activity. Fabric material may be chemically treated to improve durability (shelf life).

We acknoweldege that Schmallenberg was compared to virus in liquid, however, all results with this virus were confirmed with SARS-CoV-2, where a material control was used (and showed no significant difference from the equivalent virus in liquid).

Line 212 - The authors should state the reason why detecting SARS-CoV-2 RNA degradation is important.

Detail added.

Line 225 - What is the importance of the finding that noninfectious "fragments of SARS-CoV-2 RNA can remain on the surfaces for longer periods of time"?

Comment added – there is probably no biological significance.

Line 243 - The authors should provide an example of a commercially available Bioaerogel mask.

The authors are not aware of any commercially available Bioaerogel masks.

Reviewer 2

L31: 'cannot spread between humans well in normal situations' - please provide detail, what is a normal situation? What is 'well' relative too? Does not spread efficiently?

Corrected as per similar comment from reviewer 1

L49 - repetition of 'adopted'.

Corrected

L52 - efficacy of what?

Detail added

L53 - is it any virus? viruses spread via respiratory droplets?

Detail added

L61 - can you describe what you mean by 'salt coating of surfaces'? What kind or surface?

Corrected

L83/84 - how long where they soaked? in what volume? what temperature was the oven?

Detail added. The fabric sample size is 12 cm*10 cm. The non-woven fabric and melt-blown fabric were soaked in 200 mL salt solution until fully wetted and then transferred into the drying oven at 50°C.

L84 - watch out for tense - 'A small humidifier is...' should be 'was' instead of 'is'?

Corrected

L105 - how big were the pieces? What is 'main source material' - please clarify.

Detail has been added, as per comment from reviewer 1.

L105 - need full detail on the materials. Were they analysed at all? Supplier? size? were they sterile?

Detail added

L112 - bring the ISO method to the top. Please be clear what elements of this you followed and what you didn't. E.g. choice of virus is different.

Have added a 'disclaimer' that we did not intend to meet the standards in full – meerly use them as a guide.

L118 - 'entire surface' - what surface? how big?

More detail has been added to the methods to describe the materials.

L133 - Please describe this method.

More method details or references have been added.

Figure 2 - there was no method for SEM? Please add.

Method added. 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.

L152 - this paragraph is confusing - please clarify.

Paragraph edited.

L159 - Please use more accurate language than 'hit'

Changed to antiviral.

L162 - 'based on the ISO standards' what standards?

ISO standards numbers added.

L163 - what is a 'no material control', please clarify.

Detail added.

L168 - what does 'tested over a longer time course post-surface contact' mean?

Sentence edited for better clarity.

L211 - onwards - can you comment on the applicability of a 1log drop for RT PCR as a measure?

Detail added.

L239 - 'used as a coating'. Also reptation of word 'used'.

Sentence edited for better clarity and to avoid word repetition.

General comment for discussion - is a salt coating practical for this use? Would the humidity/moisture from breathing through a mask have an impact on the salt coating? Can you comment on the applicability of your data to the real world? At the moment it looks like data from a multiwell plate experiment is being used to suggest antiviral action of a mask.

We acknowledge that this is an *in vitro*study. We have added additional discussion has been added.

VERSION 1

Editor recommendation and comments

https://doi.org/10.1099/acmi.0.000492.v1.5

James Redfern; Manchester Metropolitan University, UNITED KINGDOM

Date report received: 28 February 2023
Recommendation: Major Revision

Comments: This is a study that would be of interest to the field and community. The reviewers have highlighted major concerns with the work presented. Please ensure that you address their comments. Please ensure all data is uploaded to a repository as per the mandatory Access Microbiology Open Data Policy. This includes data that was used to generate your figures.

Reviewer 2 recommendation and comments

https://doi.org/10.1099/acmi.0.000492.v1.4

Anonymous.

Date report received: 28 February 2023
Recommendation: Major Revision

Comments: In this manuscript the authors suggest an approach to modifying face masks to provide an antiviral property. This is likely still of interest to many, given the rise in interest in respiratory viruses. However, there are some scientific queries and clarifications that need to be addressed. Overall: In parts the writing needs clarity/more scientific language. Method needs more detail. Discussion on applicability of methods used. Specific comments: L31: 'cannot spread between humans well in normal situations' - please provide detail, what is a normal situation? What is 'well' relative too? Does not spread efficiently? L49 - repetition of 'adopted'. L52 - efficacy of what? L53 - is it any virus? viruses spread via respiratory droplets? L61 - can you describe what you mean by 'salt coating of surfaces'? What kind or surface? L83/84 - how long where they soaked? in what volume? what temperature was the oven? L84 - watch out for tense - 'A small humidifier is...' should be 'was' instead of 'is'? L105 - how big were the pieces? What is 'main source material' - please clarify. L105 - need full detail on the materials. Were they analysed at all? Supplier? size? were they sterile? L112 - bring the ISO method to the top. Please be clear what elements of this you followed and what you didnt. E.g. choice of virus is different. L118 - 'entire surface' - what surface? how big? L133 - Please describe this method. Figure 2 - there was no method for SEM? Please add. L152 - this paragraph is confusing - please clarify. L159 - Please use more accurate language than 'hit' L162 - 'based on the ISO standards' what standards? L163 - what is a 'no material control', please clarify. L168 - what does 'tested over a longer time course post-surface contact' mean? L211 - onwards - can you comment on the applicability of a 1log drop for RT PCR as a measure? L239 - 'used as a coating'. Also reptation of word 'used'. General comment for discussion - is a salt coating practical for this use? Would the humidity/moisture from breathing through a mask have an impact on the salt coating? Can you comment on the applicability of your data to the real world? At the moment it looks like data from a multiwell plate experiment is being used to suggest antiviral action of a mask.

Please rate the manuscript for methodological rigour
Satisfactory

Please rate the quality of the presentation and structure of the manuscript
Good

To what extent are the conclusions supported by the data?
Partially support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?
No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
Yes

Reviewer 1 recommendation and comments

https://doi.org/10.1099/acmi.0.000492.v1.3

Anonymous.

Date report received: 21 February 2023
Recommendation: Major Revision

Comments: General Comments: Abstract section needs to be expanded upon so that the reader gets a sense for the direction the article will take and what the authors found. For example, was there a single coating that was more effective in all test materials? Did the material type have any impact on antiviral activity given similar coatings? What else did the authors conclude from the study? How does this information add to the existing body of knowledge? Descriptions of fabric treatments in the Materials section needs to include more procedural detail so that the testing could be repeated by others, if desired. For example, what were the fabric swatch sizes, salt solution volumes, and drying oven temperature? Also, Appendix 1 needs to indicate if by "face mask" this means melt-blown fabric since nonwoven fabric is listed. It would be better to have SEM photomicrographs seen in Figure 2 show the common treatment, that being 30% KCl:NaCl (50:50 mix). It is difficult to compare the relative distribution

of salt coating from one material to the other when four different treatments are shown. The authors need to provide a rationale for testing the outer, middle, and inner layers of the "face mask" in Appendix 1 using the Schmallenberg virus. This process was not mentioned in the Materials section. Also, it should be specified if non-woven and melt-blown fabric samples seen in Figure 1 are intact 3-layers or a single separated layer. The pore sizes shown in Figure 2 appear to be much too large to ensure adequate contact of aerosolized virus particles with incorporated salt material. The Centers for Disease Control and Prevention (CDC) states that the average particle size of SARS-CoV-2 is around 0.1 micrometer (μm). Also, the minimum size of a respiratory particle that can contain SARS-CoV-2 is calculated to be approximately 9.3 μm.* Judging by the 100 μm size bar, the pore sizes in these materials, with the possible exception of MBF-30% NaCL, would be much too large for an appreciable numbers of virus particles to come into contact with the salt-coated material. Physical contact would be required to for virus inactivation via desiccation. An additional practical concern would be the breathability (ease of breathing) using a mask coated with NWF-NACL&KCL 30%. This would be questionable given that the provided SEM photomicrograph is representative of several examined fields. This type of limitation should also be discussed since this same issue is mentioned in one of their provided references.** The authors should supply the commercial source of face mask materials. *Lee BU. Minimum Sizes of Respiratory Particles Carrying SARS-CoV-2 and the Possibility of Aerosol Generation. Int J Environ Res Public Health. 2020 Sep 23;17(19):6960. doi: 10.3390/ijerph17196960 ** Rubino I, Oh E, Han S, Kaleem S, Hornig A, Lee SH, Kang HJ, Lee DH, Chu KB, Kumaran S, Armstrong S, Lalani R, Choudhry S, Kim CI, Quan FS, Jeon B, Choi HJ. 2020. Salt coatings functionalize inert membranes into high-performing filters against infectious. Specific Comments: Lines 25-29 - Suggest mentioning that the geographic restriction was most likely related to a limited geographical distribution of zoonotic sources for SARS-CoV-1 (civet cats) and MERS-CoV (dromedary camels). Line 32 - Recommend changing "well in normal situations" to "easily under normal conditions". Line 35 - The zoonotic source of SARS-CoV-2 has not been definitively established. Recommend sticking to statements indicating the relative ease by which this virus spreads from human-to-human. Lines 39-40 - Suggest describing the typical affliction brought on by hCoVs, pre- SARS-CoV-2, that being a head cold that was not geographically restricted in its incidence. hCovs were deemed responsible for ~20% of common colds until the appearance of SARS-CoV-2. Lines 52-53 - The authors should provide examples of attempts to incorporate antiviral materials (e.g., metallic nanoparticles) into facemasks and what types of masks (e.g., cloth-single vs cloth double layer) were used in these attempts. Lines 84-85 - The authors should specify the amount of salt solution applied to the biomass aerogel material. The same goes for non-woven fabrics and melt-blown fabrics soaked in salt solutions. Additionally, how big were salt-treated fabric sizes used for testing? Line 105-107 - The authors should explain the reason 2 greatly different microplates were used for testing either SARS-CoV-2 or SBV. This implies a rather large difference in sample size used to test either virus. Line 115 - Viruses are nonliving, so the word "active" should be used in lieu of "live". Line 163 - Was a material control also included? A lowering of the virus titer should be compared to an untreated material control and not to a "no material" control. It cannot be reasonably assumed that the material by itself does not possess some antiviral activity. Fabric material may be chemically treated to improve durability (shelf life). Line 212 - The authors should state the reason why detecting SARS-CoV-2 RNA degradation is important. Line 225 - What is the importance of the finding that noninfectious "fragments of SARS-CoV-2 RNA can remain on the surfaces for longer periods of time"? Line 243 - The authors should provide an example of a commercially available Bioaerogel mask.

Please rate the manuscript for methodological rigour
Poor

Please rate the quality of the presentation and structure of the manuscript
Satisfactory

To what extent are the conclusions supported by the data?
Partially support

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No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
No: Not Applicable

SciScore report

https://doi.org/10.1099/acmi.0.000492.v1.1

iThenticate report

https://doi.org/10.1099/acmi.0.000492.v1.2