



# Efficacy of copper blend coatings in reducing SARS-CoV-2 contamination

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**Abstract** SARS-CoV-2 is a highly infectious virus and etiologic agent of COVID-19, which is spread by respiratory droplets, aerosols, and contaminated surfaces. Copper is a known antiviral agent, and has resulted in successful reduction of pathogens and infections by 83–99.9% when coated on surfaces in intensive care units. Additionally, copper has been shown to inactivate pathogens such as Coronavirus 226E, a close relative of SARS-CoV-2. Here, we examine the ability of two copper blends with differing compositions to inactivate SARS-CoV-2 virus at different time points. Copper Blend 2 (75.07% pure copper) was found to significantly reduce (over 50%) the viability of SARS-CoV-2 at 5 min of contact, with at least 98% reduction in recovered virus at 20 min (vs. plastic control). However, Copper Blend 1 (48.26% pure copper), was not found to significantly reduce viability of SARS-CoV-2 at any time point when compared to plastic. This may indicate that there is an important percentage of copper content in materials that is needed to effectively inactivate SARS-CoV-2. Overall, this study shows that over the course of 20 min, coatings made of copper materials

can significantly reduce the recovery of infectious SARS-CoV-2 compared to uncoated controls, indicating the effective use of copper for viral inactivation on surfaces. Furthermore, it may suggest higher copper content has stronger antiviral properties. This could have important implications when short turnaround times are needed for cleaning and disinfecting rooms or equipment, especially in strained healthcare settings which are struggling to keep up with demand.

**Keywords** SARS-CoV-2 · COVID-19 · Copper · Copper blend · Viral inactivation · Antiviral agent

## Introduction

SARS-Coronavirus-2 (SARS-CoV-2), the etiologic agent of COVID-19, is primarily spread by respiratory droplets, but also through contaminated surfaces and aerosols (Govind et al. 2021; Jin et al. 2020; Kraay et al. 2020; Marquès and Domingo 2021; Ren et al. 2020; van Doremalen et al. 2020; Ye et al. 2020; Zuo et al. 2020). To prevent spread of the virus, emphasis was initially put on basic public health practices, including isolating, hand washing, and mask wearing (CDC, 2021, 2020; Gostin et al. 2020; Johns Hopkins University, 2021). These mitigation efforts were largely aimed at stopping the spread of droplet-associated SARS-CoV-2 viral particles both in the context of human-to-human transmission, as well as surface contamination. Even with

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these efforts, SARS-CoV-2 spread across the globe, indicating a need for additional research on transmission prevention.

Recent research suggests that respiratory droplets from breathing, speaking, coughing, and sneezing can be carried distances greater than 2 m (Bahl et al. 2020). These droplets often land on high-touch surfaces (e.g., doorknobs, elevator buttons, cell phones), which allows infectious virus to be mechanically transmitted to the mouth, nasal mucosa, or conjunctiva of others. Droplet spread and pathogen survival depend on factors including surface porosity, temperature, ventilation, and relative humidity, but some droplet-associated viruses remain infectious on common surfaces for days or weeks (Aboubakr et al. 2021; Bueckert et al. 2020). Infectious pathogens have been found to survive and remain infectious for extended periods of time on high-touch surfaces, including those in healthcare settings (e.g., bed rails, IV poles) (CDC, 2021; Mantlo et al. 2020; Otter et al. 2013; Zerbib et al. 2020). Specifically, SARS-CoV-2 has been found to be viable on non-porous surfaces for days to weeks (Biryukov et al. 2020; Chin et al. 2020; Liu et al. 2021; Riddell et al. 2020; van Doremalen et al. 2020). Additionally, SARS-CoV-2 was found to have substantial transfer from non-porous solids to artificial skin through light touch when the droplet was wet (13–16% virus transfer) as well as after it had evaporated (3–9% virus transfer) (Behzadinasab et al. 2021a). Consequently, disinfection of high-touch surfaces, particularly during a pandemic and in healthcare settings, provides an opportunity to decrease the spread of deadly pathogens (Bahl et al. 2020; Otter et al. 2016).

Numerous studies have demonstrated the antibacterial and antiviral activity of copper against a wide range of pathogens including *E. coli*, Influenza A, Norovirus, SARS-CoV-1, herpes simplex, Junin, HIV-1, poliovirus, monkeypox, and Marburg and Ebola viruses (Champagne et al. 2019; Cortes and Zuñiga 2020; Govind et al. 2021; Grass et al. 2011; Han et al. 2005; Imai et al. 2012; Mantlo et al. 2020; Manuel et al. 2015; Michels et al. 2015; Montero et al. 2019; Noyce et al. 2007; Rakowska et al. 2021; Rosenberg et al. 2018; Rózańska et al. 2017; Warnes et al. 2012; Wilks et al. 2005). In addition, laboratory results have led to the use of copper materials in clinical trials in healthcare facilities and community centers (Casey et al. 2010; Colin et al. 2018;

Hinsa-Leasure et al. 2016; Ibrahim et al. 2018; Poggio et al. 2020; Zerbib et al. 2020; Schmidt et al. 2012), with hospital intensive care units containing copper-coated appliances reporting 83–99.9% reduction in the burden of pathogens and infections (Montero et al. 2019; Salgado et al. 2013). The exact mechanism behind the ability of copper to inactivate or kill pathogens is believed to differ between pathogen types (Festa and Thiele 2011; Manuel et al. 2015; Rosenberg et al. 2018; Warnes et al. 2012, 2015). For viruses, the proposed mechanism is that copper ions disrupt viral envelopes, prevent cellular respiration, produce free radicals, and destroy the DNA/RNA of microbes when in contact with copper surfaces (Rakowska et al. 2021).

Copper inactivation of Coronavirus 226E, another common respiratory virus and close relative of SARS-CoV-2, has been successful, with inactivation observed in 40 min or less (Warnes et al. 2015). Recent studies by Behzadinasab et al. (2020), Hutasoit et al. (2020), and Mantlo et al. (2020) examined Cu<sub>2</sub>O/PU film, 3D-printed copper-coated surfaces, and cold-spray copper coating, respectively, for SARS-CoV-2 inactivation capabilities. Behzadinasab et al. (2020) reported 99.99% inactivation after 1-h incubation while Hutasoit et al. (2020) and Mantlo et al. (2020) found 96% and 99% inactivation after 2 h. Another study by Behzadinasab et al. (2021b) studied two transparent surface coatings, PDA/Cu and PDA/Cu<sub>2</sub>O, which also found 99.98% and 99.88% inactivation of SARS-CoV-2 after 1-h incubation, respectively. However, viability of the novel SARS-CoV-2 virus immediately after contact with copper materials has not been adequately tested. It may be that, coating surfaces with copper infused materials can more rapidly inactivate SARS-CoV-2 than we currently understand. Accordingly, in this study, we evaluated the inactivation of SARS-CoV-2 virus upon contact with two copper blend coatings from 1 to 20 min.

## Materials and methods

### Viral strains and cell lines

Vero E6 (ATCC® CRL-1586™) cells were cultured in 1× Dulbecco's Modification of Eagle's Medium with 4.5 g/l glucose supplemented with 1%

L-glutamine (DMEM; Corning, Corning, NY) and 10% (v/v) fetal bovine serum (FBS; Corning, Corning, NY). Vero E6 cells were grown in T175 flasks and passaged when the cells were 90% confluent.  $1.2 \times 10^6$  cells were plated in 6-well plates overnight before use.

The SARS-CoV-2 Chile strain was obtained from BEI (NR-52439). One hundred  $\mu\text{l}$  was added to a confluent T75 flask of Vero E6 cells in serum-free media (SFM) with antibiotics. Forty-eight hours later, 100  $\mu\text{l}$  of supernatant was added to a second flask with uninfected cells. If cytopathic effects were observed after 48 h, the supernatant was removed and the flask containing the remainder of the cells were placed in a freezer for 5 min. The flask was then thawed, and the cells were collected using 5 ml of new media. The cell lysate was mixed with supernatant and centrifuged at  $500 \times g$  for 5 min before aliquoting.

#### Surfaces tested

Two distinct copper blends were formulated by Alloi, with Copper Blend 1 containing 48.26% pure copper and Copper Blend 2 containing 75.07% pure copper. The composition of each blend's inputs is characterized in Table 1. To create these materials, copper and zinc were ordered from a third-party and then mixed with a non-metal binder (styrene resin sold under COR75-AQ-010L [INTERPLASTICS Unsaturated Polyester Resin product code: SIL94BA-990]) and catalyst (Methyl ethyl ketone peroxide [MEKP]). Both blends consist of metallic components as well

as the binder, to make the material as a whole not conductive, and the catalyst, to harden the binder. All materials were measured using a scale with an uncertainty of  $\pm 0.01$  g. The two copper blends were then used to completely cover pieces of 2 cm  $\times$  2 cm polycarbonate plastic sheets with 0.1 mm thickness using cold-spray technology. These materials were then brought to the George Washington University Nanofabrication and Imaging Center and imaged using the GEI Teneo LV scanning electron microscope (SEM) with an EDAX Octane Pro detector for elemental analysis. SEM was done at  $5000 \times$  and energy-dispersive x-ray spectroscopy (EDS) was performed at 30 kV for 100 s showing Copper Blend 1 to be mainly copper (Cu) 41.61% and zinc (Zn) 14.59%, and Copper Blend 2 to be predominately Cu 100%. Further analysis of these coated materials by SEM and energy-dispersive X-ray spectroscopy (EDS) is shown in Fig. 1 and Table 2. The copper blend-coated samples arrived in sealed bags and were tested as is. The uncoated plastic samples arrived with a thin sheet of plastic adhesive covering one side. The plastic adhesive was removed with 70% ethanol and allowed to dry before experimentation. The components of the copper blends were unknown to the researchers at the time of experimentation; however, composition was revealed to the researchers after analysis of results.

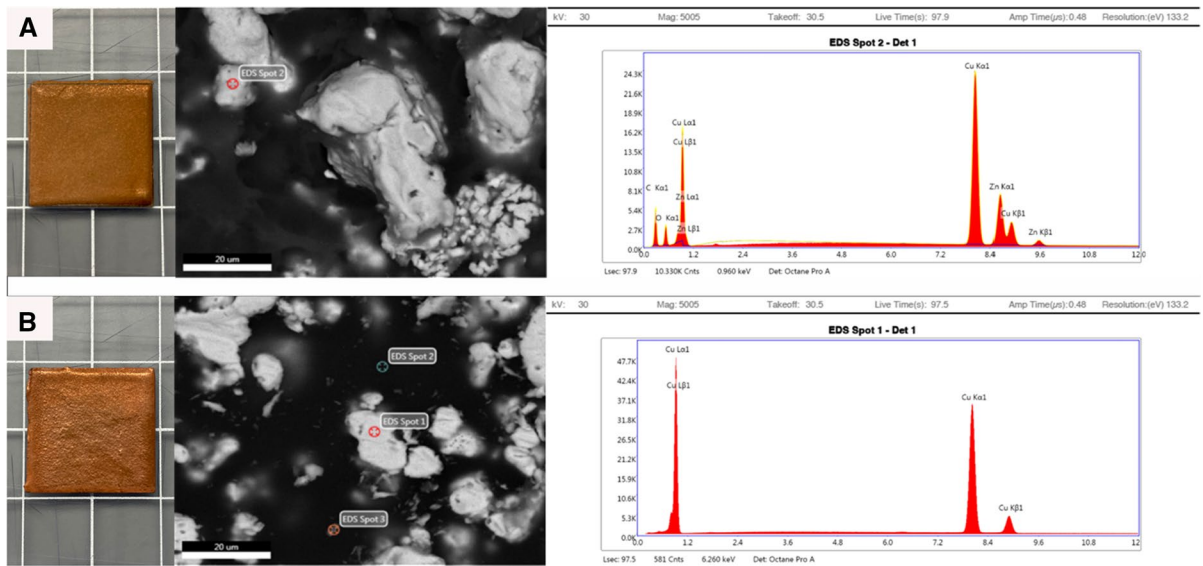
#### Infectivity assay for SARS-CoV-2

Viral stock solution was thawed and placed in ten 1  $\mu\text{l}$  droplets on two different copper blend-coated

**Table 1** Composition of the inputs for Copper Blend 1 and 2 by weight and percent of total weight

Material	Copper Blend 1		Copper Blend 2	
	Weight (g)	Percent of total weight	Weight (g)	Percent of total weight
Binder (styrene resin [COR75-AQ-010L])	45.0	24.13%	45.0	24.13%
Catalyst (methyl ethyl ketone peroxide [MEKP])	1.5	0.80%	1.5	0.80%
Pure copper (without brass)	90.0	48.26%	140.0	75.07%
Brass (70% copper/30% zinc)	50.0	26.81%	0.0	0.00%
Total weight	186.5	–	186.5	–
Zinc <sup>a</sup>	15.0	8.04%	0.0	0.00%
Total copper (pure copper + brass) <sup>a</sup>	125.0	67.02%	140.0	75.07%

<sup>a</sup>Zinc weight and percent of total weight were calculated based on brass content. Total copper weight and percent of total weight were calculated based on brass and pure copper content



**Fig. 1** Photographs of the appearance of: **A)** Copper Blend 1 from left to right: tested material in a 2×2 cm square, scanning electron microscopy (SEM) done at 5000x, energy-dispersive X-ray spectroscopy (EDS) at 30 kV for 100 s showing composition of Copper Blend 1 Spot 2 to be predominately copper (Cu) 41.61% and zinc (Zn) 14.59%; **B)** Copper Blend

2 from left to right: tested material in a 2×2 cm square, SEM done at 5000x, EDS at 30 kV for 100 s showing composition of Copper Blend 2 Spot 1 to be predominately Cu 100% (background analysis of Copper Blend 2 Spot 2 and Spot 3 were predominantly carbon, 57.64% and 43.18%, respectively)

**Table 2** Composition of Copper Blend 1 and 2 products by percent weight using energy-dispersive x-ray spectroscopy (EDS)

Element	Copper Blend 1 (spot 2) <sup>a</sup>		Copper Blend 2 (spot 1) <sup>a</sup>	
	Weight %	Error %	Weight %	Error %
Copper	41.61	1.34	100	0.99
Zinc	14.59	1.45	–	–
Carbon	33.98	8.96	–	–
Oxygen	9.82	9.90	–	–

<sup>a</sup>EDS spot locations are displayed in Fig. 1 above

surfaces to simulate respiratory droplet contact (Lindley et al. 2013; Warnes et al. 2015). The distinct 1 μl droplets were used instead of one 10 μl droplet to allow for more direct contact with the copper blend surfaces due to the surface tension of the solution (Mantlo et al. 2020). The virus was removed from the test surfaces by washing with 490 μl EMEM supplemented with 1% penicillin, streptomycin, amphotericin B and non-essential nucleic acids after incubation at room temperature for various time points:

1 min, 5 min, 10 min, and 20 min. The virus and media mixture was assayed for infectious virus survival using a plaque assay, quantified as plaque forming units (PFU). Serial dilutions of the virus mixture were prepared in infection medium before 200 μl aliquots were plated onto confluent monolayers of Vero E6 cells in Corning® 6-well plates. After 1 h, a 1:1 agar and EMEM overlay was added and plates were incubated at 37 °C and 5% CO<sub>2</sub> for 72 h. Following incubation, the plates were fixed with 10% (w/v) formaldehyde, stained with 0.5% Crystal Violet and allowed to dry before plaques were counted.

### Statistical analyses

All statistical analyses were performed in R Studio (version 1.3.1093) with base R (version 3.6.3) and significance was assessed at the  $\alpha=0.05$  level. We first wanted to compare whether the recovery of viable SARS-CoV-2 was differentially affected by incubation on three different materials (Copper Blend 1, Copper Blend 2, and plastic) at four different time points. Median rank titers (Log PFU/ml) across the three materials were compared using the

Kruskal–Wallis non-parametric analysis of variance (kruskal.test) after determination that data were not distributed normally (shapiro.test,  $p < 0.05$ ). When warranted, post-hoc tests for pairwise differences were performed using the Dunn Test (dunnTest, package FSA). We next wanted to compare the performance of each blend compared to the baseline material of plastic. We took the difference of recovered viral titer (log PFU/mL) for each trial-blend combination and calculated the percent reduction from the corresponding trial’s plastic control at each time point. We then used a t-test to determine whether these calculated differences were significantly greater from select null values; namely, 50%, 75%, and 98%. A rejection of the null in this case would indicate that an blend had at least or greater reduction than the null value tested.

**Results**

Comparison of recovered titer from each type of material

At 1-min exposure time, there was no significant difference in the recovered viral titer (PFUs/mL) among

the three materials. At 5, 10, and 20 min contact with Copper Blend 2, there were significantly lower recovered titers of SARS-CoV-2 than both Copper Blend 1 and plastic. The viral titer recovered from Copper Blend 1 was not significantly different from the plastic control at any time point measured (Fig. 2).

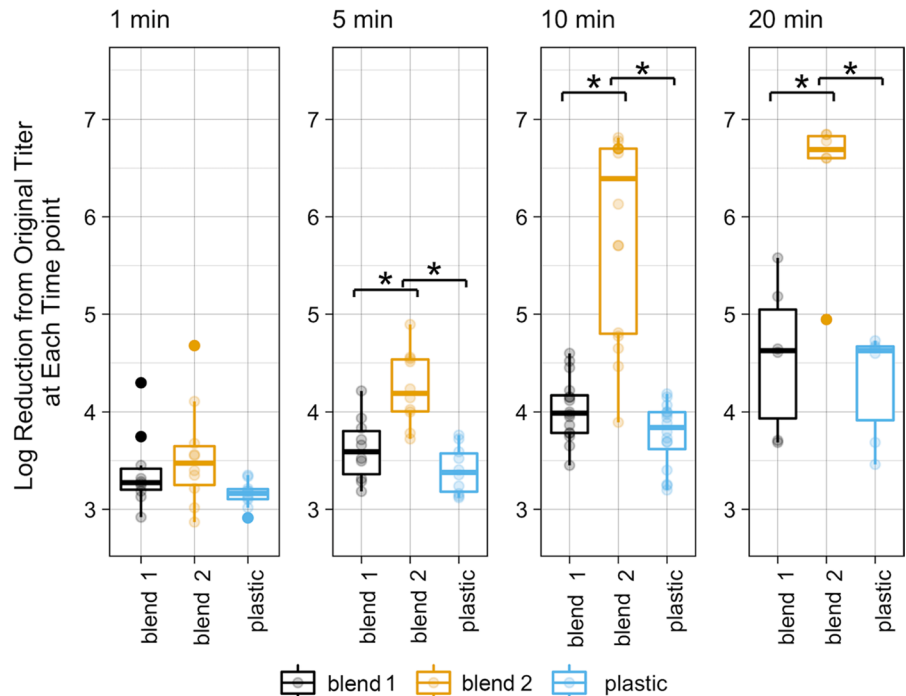
Percent reduction of recovered titer of Copper Blend 1 or Copper Blend 2 compared to the plastic control

When the recovered viral titer of each copper blend was compared to that of the plastic control, Copper Blend 1 did not result in a SARS-CoV-2 titer reduction that was significantly greater than 50% at any timepoint. However, for Copper Blend 2, the percent reduction in titer compared to the plastic control was significantly higher. Reduction was significantly greater than a maximum of 50% at time point 5 min, 75% at 10 min, and 98% at 20 min (Fig. 3).

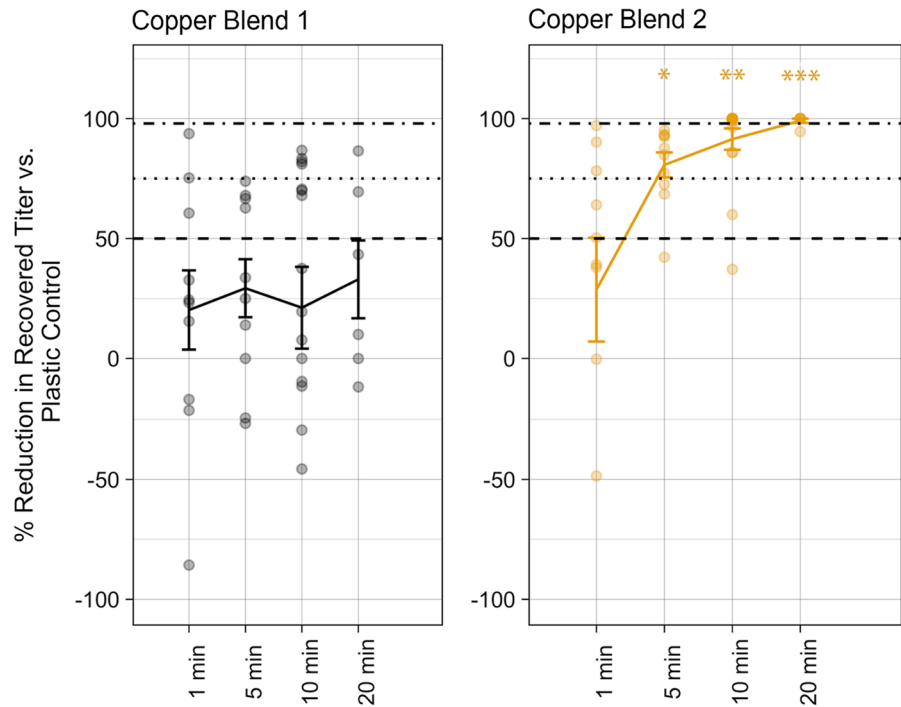
**Discussion**

Basic infection prevention and control practices are important to prevent the rapid spread of novel viruses, such as SARS-CoV-2. Part of this strategy

**Fig. 2** At 5, 10, and 20 min, contact with Copper Blend 2 produced significantly (\*) lower titers (Log PFU/ml) than exposure to either Copper Blend 1 or plastic ( $p < 0.05$ ). Presented are data points and boxplot data summaries



**Fig. 3** For each copper blend material, points represent percent reduction from matching plastic control. Lines represent the mean percent reduction with  $\pm$  standard error. \*Indicates significantly greater reduction than 50%, \*\*Indicates significantly greater reduction than 75%, \*\*\*Indicates significantly greater reduction than 98%



includes timely and consistent disinfection of surfaces, especially in high-trafficked spaces where respiratory droplets may contain viruses capable of surviving for extended periods of time on commonly used surfaces. Reducing environmental contamination is particularly important during pandemics and in health care settings. Previous studies found copper is an effective option to combat fomite transmission both in laboratory and in clinical settings (Behzadinab et al. 2020; Hutasoit et al. 2020; Mantlo et al. 2020; Warnes et al. 2015). This current study sought to build on the growing body of knowledge regarding the uses of copper and copper materials as deterrents of surface-mediated transmission of viruses.

Our results indicate that contact with a high copper-content material inactivated SARS-CoV-2 as compared to a plastic control in a time-dependent manner. There was no significant difference in recovered titer between Copper Blend 1, Copper Blend 2, and the plastic control after 1 min of contact, suggesting a minimum time of contact is needed for efficacy. Copper Blend 2 begins to significantly reduce the viability of SARS-CoV-2 at 5 min of contact with at least 98% reduction in recovered virus at 20 min of contact.

Copper Blend 2 is 75.07% pure copper composition by weight compared to Copper Blend 1, which has a pure copper composition of 48.26%. The difference in recovered virus between the two suggests that higher copper content may have stronger antiviral properties. It is important to note that Copper Blend 1 also contained brass, which is a copper alloy with 70% copper/30% zinc, meaning Copper Blend 1 has a total copper content of 67.02%. Our results are similar to a study that demonstrated copper was efficacious at inactivating a human Alphacoronavirus, HuCoV-229E (Warnes et al. 2015). Pure copper and 90% copper materials were able to inactivate HuCoV-229E in 20 min (Warnes et al. 2015). Interestingly, inactivation efficiency began to decrease when the pure copper content was reduced to 85%. As SARS-CoV-2 is more stable on surfaces than HuCoV-229E, determining the efficacy of a specific material's copper content is critical to evaluate further (Rabenau et al. 2005; van Doremalen et al. 2020).

Other studies have reported greater than 96% inactivation of SARS-CoV-2 after an hour of contact with different copper formulations (Behzadinab et al. 2020; Hutasoit et al. 2020; Mantlo et al. 2020). Accordingly, our experimental limit of 20 min of contact between the viral inoculum and copper blends



may offer a conservative estimate of the ultimate effectiveness of such materials. We cannot rule out the possibility that Copper Blend 1 may have more effectively inactivated SARS-CoV-2 or that Copper Blend 2 may have achieved even greater reduction at contact times exceeding 20 min. However, the effectiveness of Copper Blend 2 at such a short interval may prove beneficial during periods of high community transmission and healthcare stress, when shorter turnaround times for cleaning and disinfecting rooms or equipment is needed to keep pace with demand.

In conclusion, this study has found that over the course of 20 min, copper blend coatings can significantly reduce the recovery of infectious SARS-CoV-2 compared to uncoated controls. Our results indicate the continued use of copper as a viral inactivator for surfaces at-risk for contamination. Furthermore, it may be that there is an important percentage of copper content in materials that is needed for effectiveness against SARS-CoV-2.

**Author contributions** AG performed the laboratory work and wrote the main manuscript text, KEK co-lead the writing of the manuscript, RCC led the data analysis, CNM conceptualized the study and secured funding. All authors read and approved the final manuscript.

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#### Declarations

**Conflict of interest** The authors have declared that no competing interests exist.

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