Reduction of SARS-CoV-2 Infectious Titers by Direct Contact with Cuprous-Oxide Impregnated Face Masks External Layers

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Abstract

SARS-CoV-2 can remain viable on the protective face masks surface for several days. Mask touching, reuse and disposal occurs frequently, leading to increased risk of cross-contamination, infection and further transmission. Cuprous-oxide has potent virucidal properties. We determined the capacity of surgical face masks (type IIR) made with nonwoven fabric impregnated with cuprous-oxide microparticles (Test Fabric), to inactivate SARS-CoV-2 when in direct contact with the virus. The Test Fabric reduced the infectious titers of SARS-CoV-2 by 0.73, 3.02 and 4.19 log10 within 5, 30 and 60 minutes, respectively. In contrast, the infectious titers of the virus were reduced by Control Fabric by 0.24, 0.67 and 0.97 within 5, 30 and 60 minutes, respectively. The reductions were significantly higher in the Test Fabric than in the Control Fabric (0.49, 2.35 and 3.22 log difference, accordingly), reaching a statistically significant difference after 5 minutes ($p < 0.01$). The mask filtration properties were not affected by the presence of the cuprous oxide microparticles. We conclude that the use of cuprous-oxide containing face masks in the external layers of respiratory face masks may significantly reduce the risk of SARS-CoV-2 cross-contamination, transmission and infection, due to masks handling and disposal, especially when used by the general population.

Introduction

The current ongoing pandemic caused by the highly pathogenic novel human coronavirus,[1] named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has infected more than 125 million individuals and caused more than 3.3 million deaths worldwide (https://covid19.who.int/, accessed May 05, 2021). The main mode of the virus transmission is through aerosol particles and droplets generated during coughing, sneezing or talking by symptomatic patients and from asymptomatic individuals, even before or without the onset of symptoms.[2,3] No significant differences were found in viral burden between symptomatic and asymptomatic individuals,[4] and thus asymptomatic individuals may unknowingly contribute significantly to the spread of the virus. One of the major effective measures taken worldwide to stop the transmission of the virus is the use of protective face masks by the general population.[5]

Copper, and cuprous-oxide in particular, have potent virucidal properties,[6] including against SARS-CoV-2.[7] A platform technology that impregnates different textiles with cuprous-oxide microparticles was developed, endowing them with wide spectrum biocidal properties.[8,9] N95 respiratory masks in which the external layers were made with nonwoven fabric impregnated with the cuprous-oxide microparticles reduced by more than 99.9% the infectious titers of Human Influenza A (H1N1) and Avian Influenza Virus (H9N2) virions that remain on the mask.[10] The high capacity of the cuprous-oxide impregnated fibers and fabrics to neutralize 12 additional different pathogenic viruses was demonstrated in separate studies.[11,12]

In the current study, we examined the capacity of the external layers of 3-ply respiratory masks, made of nonwoven cuprous-oxide impregnated fabric, to reduce the infectious titers of SARS-CoV-2 when in direct

contact with the fabric.

Materials And Methods

The test material was spunbond nonwoven polypropylene fabric containing ~ 3% cuprous-oxide microparticles (MedCu Technologies, Ltd. Figure 1), hereafter referred as the Test Fabric.

The cuprous oxide particles were characterized by x-ray diffraction (XRD) using an x-ray diffractometer (Y-2000) with Cu Kα radiation (λ = 1.5418 Å). A scan efficiency of 0.1°·S-1 was applied to record the powder patterns in the range of $0° \leq 2\theta \leq 60°$. Three peaks at 2θ = 29.61°, 36.49° and 42.37° were indexed to (110), (111), and (200) planes of the cubic phase with lattice constant α = 0.4266 nm, which is in accordance with the spectrum for Cu_2O in JCPDS–International Centre for Diffraction Data (PDF, Powder Diffraction File, No. 05–0667, 1996). Impregnation of cuprous oxide microparticles in the polypropylene fibers was achieved by adding the cuprous oxide microparticles to the polypropylene during the master batch preparation stage.[8] The concentration of the cuprous oxide microparticles in the fabric was based on previous studies that demonstrated that this concentration endows the fabric with wide spectrum biocidal properties (e.g. ref[8,13]). As a negative test control material, the same spunbond polypropylene fabric without cuprous-oxide was used, hereafter referred as Control Fabric.

After being UV sterilization for 1 hour, 20 mm by 20 mm pieces of Control and Test Fabrics were aseptically placed together in separate vial containers (final weight of 0.20 ± 0.005 g per tested sample), hereafter referred to as Control or Test Fabric specimens, respectively.

As the virus source, -80°C cryopreserved SARS-CoV-2 clinical isolate was used. The stock virus titer was determined by infecting Vero-E6 (CRL-1586™; American Type Culture Collection) plated cells grown in Gibco phosphate-free, high glucose DMEM media (catalog number: 11971025) containing 10% fetal bovine serum (FBS) and determining the plaque forming units (PFU)/ml.

The determination of the direct contact virus inactivation of the test items was performed as follows according to the ISO Standard 18184:2019 -Textiles — Determination of antiviral activity of textile products: 100 µl of the virus stock suspension were placed onto each test specimen, making sure all the liquid was thoroughly absorbed. The vial containers were then tightly closed and incubated at of 25°C for 5, 30, or 60 min (triplicate Control or Test Fabric specimens per time point). After the respective incubation time, 10 ml of Earle's Balanced Salt Solution (EBSS, Gibco) were added to each container; the containers were closed and agitated for 60 seconds by vortexing. The virus suspensions were collected. From each of the recovered eluent suspensions after vortexing, 2-times sequential dilutions were prepared using phosphate free DMEM media.

For potential deactivation of the virus by possible eluting elements from the Control or Test Fabric specimens (i.e. not by direct contact inactivation), the following control was performed: 10 ml of EBSS were added to 3 Control Test specimens and to 3 Test Fabric specimens placed in the vial containers. The containers were then closed, vortexed for 60 s, and 5 ml of the solution from each vial container were

transferred to new tubes. 50 µl of virus suspension were then added to each tube. The tubes were kept at 25°C in the CO_2 incubator for 30 min.

The infectivity of the virus recovered from the Control or Test Fabric specimens were then determined by conducting a plaque assay using Vero-E6 cells plated in 96 well culture plates 12–24 hours in advance, and grown to 80–90% confluency. Briefly, the cells were washed once with PBS and then 100 µl of sequential dilutions the above-described suspensions were added to each well. The cells inoculated with the virus were incubated for 1 hour at 37°C in 5% CO_2 incubator, with gentle shaking every 15 minutes. After 1 hour of absorption, 100 µl of a semi-solid maintenance solution preparation (1% methylcellulose in DMEM) were added to each well. The plates were incubated for 3 days at 37°C in 5% CO_2 incubator. The number of plaques formed was monitored every day. After 72 hours of incubation, when the formation of plaques was obvious, 50 µl of 2% paraformaldehyde were added per well in order to fix the cells. The supernatants were discarded after 30 minutes, and 30 µl of crystal violet were added per well. After 2 minutes, the cells were washed 5 times with water. The number of plaques was determined by using an inverted microscope.

The plaque forming units (PFU) per ml were calculated using the following formula:

PFU/ml = number of plaques counted/(dilution factors x inoculation volume).

Each PFU/ml was converted to log 10 and the average of the Initial viral load (Log10) and Output viral load (Log10) were then determined.

The log10 Reduction was calculated in the following manner:

Log10 Reduction = Average Initial viral load (Log10) – Average Output viral load (Log10).

The percent reduction of the infectious titers was calculated as follows:

Percent reduction from Initial titer = 100-[(Mean final titer / Mean initial titer)*100]

A t-test was performed to compare the means of log reductions from direct contact inactivation as well as the total reduction for statistical significance between control and test fabrics. A p-value of less than 0.05 was considered a statistically significant difference.

The amount of copper eluting from the mask into human dermatomed skin was determined in an independent laboratory (Dermal Technology Laboratory Ltd., Med IC4, Keele University Science and Business Park, Keele, Staffordshire, UK) according to the OECD Test No. 428: Skin Absorption: In Vitro Method. Regular surgical face masks without copper were used as negative controls. The surgical face mask materials (triplicate samples) were applied to the surface of the skin in static diffusion cells and the skin was otherwise left unoccluded for an exposure period of 24 hours. Various skin compartments of a typical OECD 428 study[14] were collected and analyzed for copper by Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES).

The amount of copper that eluted from the masks into the air under simulated breathing conditions was determined by an independent lab (Nelson Laboratories, Inc. 6280 S. Redwood RD., Salt Lake City, UT, USA). To simulate human inhalation, air was pulled under vacuum of 1 CFM through a capsule HEPA filter and a Concha Therm III® unit, which can heat and humidify air. The temperature of the test was maintained at 37 ± 2°C and > 90% relative humidity. In addition, a 4 ± 2% concentration of carbon dioxide was introduced into the air stream. The air was pulled through the test sample and releasable particles were drawn through a Pall® zefluor 3 µm PTFE membrane, with a particle collection efficiency of > 99.996% of 0.3 µm diameter. After 5 hours of exposure to simulated inhalation, the test was stopped and the collection membranes were removed and the amount of copper was determined by ICP-OES. The test was done in triplicates. Regular surgical masks without copper were used as negative controls. As a positive control, 0.1 grams of cuprous oxide powder were added to 100 ml of USP water and 1 ml of the mixture was inoculated onto a PTFE particle collection membrane and the solution was allowed to dry.

The filtration properties of the masks were determined according to EN 14683 (European standard for face masks) by an independent lab (TUV SUD Products Testing Co., Ltd. B-3/4, No. 1999 Du Hui Road, Minhang District, Shanghai, China), which included determination of Bacterial Filtration Efficiency (BFE), Differential Pressure and Synthetic Blood Penetration tests.

Results

The virus infectious titers were not reduced following the exposure of the virus to the EBSS medium that was in contact with the Control and Test specimens (Table 1). The obtained mean titers were somewhat higher than the mean value obtained from the virus suspensions that were not mixed with the tested fabrics. In order to account for the variability of the obtained initial titer, considering that Replicate 3 of the original virus suspension was an outlier, in the following calculations we referred to the initial viral titer as the average of all 8 wash-out measurements obtained, excluding the outlier, i.e. 232,000 PFU/ml, and the mean log 10 of all 7 wash-out measurements obtained is 5.35 log10.

Table 1
Wash-out control results

Virus Suspension Replicate	Viral titre (PFU/ml)	Mean ± SD (PFU/ml)
Original 1	208,000	165,330.3 ± 73,900.1
2	208,000	
3	80,000	
Original + washing 1	192,000	218,660.7 ± 46,180.8
out Test Fabric 2	192,000	
3	272,000	
Original + washing 1	272,000	234,660.7 ± 40,260.6
out Control Fabric 2	272,000	
3	240,000	

Following the direct contact of the virus suspension with the Test Fabric specimens, the infectious titers of SARS-CoV-2 was reduced by 0.73, 3.02 and 4.19 log10 within 5, 30 and 60 minutes respectively (Table 2).

Table 2
Virus titers following direct contact with the Test Fabric

Contact Time (min)	Replicate	Calculated titre (PFU/ml)	Mean titre (PFU/ml ± SD)	Average Log10 Viral Load	Log10 reduction from initial titer[†]
5	1	52,000	42,667.0 ± 8326	4.62	0.73
	2	36,000			
	3	40,000			
30	1	5,000	2,333.7 ± 2516	2.33	3.02
	2	2,000			
	3	1[‡]			
60	1	3,000	1000.7 ± 1731	1.16	4.19
	2	1			
	3	1			

[†] average of all data obtained in Table 1, reflecting the titer of the initial viral inoculum (232000 PFU/ml = 5.365 log10)

[‡] Lower limit of detection

The infectious titers of the virus that was in contact with the Control Fabric were also reduced in a time dependent manner, but to a significantly lower extent (Table 3). As depicted in Fig. 2, the reductions of the infectious titers were significantly higher in the Test Fabric than in the Control Fabric (0.49, 2.35 and 3.22 log difference after 5, 30 and 60 minutes, respectively), reaching a statistically significant difference already after 5 minutes ($p < 0.01$).

Table 3
Virus titers following direct contact with the Control Fabric

Contact Time (min)	Replicate	Calculated titre (PFU/ml)	Mean titre (PFU/ml ± SD)	Average Log10 Viral Load	Log10 reduction from initial titer†
5	1	192,000	149,330.3 ± 36,950	5.11	0.24
	2	128,000			
	3	128,000			
30	1	80,000	53,330.0 ± 24440	4.68	0.67
	2	48,000			
	3	32,000			
60	1	48,000	29,330.3 ± 16650	4.38	0.97
	2	24,000			
	3	16,000			

† average of all data obtained in Table 1, reflecting the titer of the initial viral inoculum (232000 PFU/ml = 5.365 log10)

No significant differences were found in the amount of copper detected in the unwashed, digested skin samples, and in the washed and tape stripped skin samples exposed to the cuprous oxide containing masks and the control masks (1.62 ± 0.44 and 1.4 ± 0.8 µg, respectively; p = 0.64), as determined by a typical OECD Test No. 428: Skin Absorption: In Vitro Method. The amount of copper that eluted from the masks during 5 hours of simulated breathing conditions was 0.09 pg/m^3.

The cuprous oxide impregnated masks passed successfully all three EN 14683 tests, achieving ≥ 99.8% in the Bacterial Filtration Efficiency (n = 5) test, 30.1 Pa/cm^2 in the Differential Pressure test (n = 5) and no penetration of synthetic blood (n = 13).

Discussion

The capacity of copper to neutralize readily coronaviruses has been previously demonstrated.[15] More recently it has been found that the SARS-CoV-2 can remain viable on surfaces between hours and days, depending on the inoculum shed and environmental conditions.[16] While on plastic, stainless steel, and cardboard, the median half-life of survival for the SARS-CoV-2 is 6.81, 5.63 and 3.46 hours, respectively, on metallic copper it is 0.77 hours; less than in the aerosols (1·09).[16] Copper ions released from the metallic copper and the generation of reactive oxygen species (ROS) was shown to be involved in the inactivation of the viruses that come in contact with the copper surfaces.[15] In the above-described antiviral nonwoven fabrics, the active copper form is already in the oxidized (activated) form (cuprous-

oxide). This is one very significant step closer to releasing the active copper ions that damage the viruses, and hence the faster inactivation of the virions, as opposed to the slower inactivation observed with pure copper.[16]

Surgical masks, N95 and other respiratory masks, are now being used widely not only by healthcare and first responders, but by the wide public, in view of the current COVID-9 pandemic. Disturbingly, SARS-CoV-2 virus can be retrieved from the surface of regular face masks even after 7 days of exposure of the mask to the virus.[17] While it is recommended that respiratory face masks be used until they are soiled,[18] in reality the general population reuses their face masks even for several days, sometimes until the masks are disintegrated. It has already been demonstrated that face masks and respirators can become contaminated with viral pathogens following their prolonged use,[19–21] and that mask and face touching is a frequent habit.[22]

The presence of antiviral nonwoven fabrics, in the layer in contact with the face and in the external layer of the mask, may significantly reduce the risk of cross contamination during mask handling and disposal. The internal layer is especially relevant regarding asymptomatic individuals, who unknowingly contaminate their masks. Following the mask removal, they may contaminate their hands or gloves, and then unintentionally contaminate other high touch surfaces, such as door handles and elevator buttons. These surfaces may be touched by unexposed individuals, who then can become infected.

The cuprous-oxide impregnated nonwoven fabric has been safely used for years in adult diapers and antimicrobial wound dressings,[23,24] which have been cleared by the FDA and other regulatory bodies. Their safety in respiratory face masks was also demonstrated.[10] No copper eluted from the copper containing face mask even when the fabric had been in continuous contact with human skin for 24 hours. It was also found that the amount of copper that eluted to the air from the masks during 5 hours under simulated breathing conditions was ~ 100,000-fold lower than the respiratory copper permissible exposure limit (PEL) set by the USA Occupational Safety and Health Administration (“OSHA”). The lowest observed-adverse-effect levels (“LOAELs”) for chronic copper inhalation exposure was determined to be 0.64 mg/m^3. The copper levels eluted during the simulated breathing test, from the copper containing masks, was 0.09 pg/m^3. This is a tiny fraction (1,000,000-fold less) of the copper LOAEL. The masks passed successfully the EN 14683 tests, achieving the type IIR surgical mask parameters threshold, indicating that the presence of the cuprous oxide microparticles did not interfere or affected the filtration properties of the masks. Importantly, the masks passed all the safety biocompatibility tests, showing that they do not cause any skin sensitization or skin irritation. This is in accordance with as the extensive safe use of cuprous-oxide wound dressings and diapers by thousands of individuals.[22,23]

Conclusions

Face masks are now widely used by the general population in view of the ongoing COVID-19 pandemic. Masks may become contaminated with the SARS-CoV-2 virus, including unknowingly by asymptomatic

individuals. Since the virus may remain infectious on the masks for several days, and since masks are being reused by the general public for several days, viral cross-contamination and increased risk of viral infection due to the improper mask handling and disposal, may occur. Thus, the use of cuprous-oxide containing face masks in the external layers of the masks, which can significantly readily reduce the viral infectious titers, may contribute to reduction of viral cross-contamination, transmission and infection.

Declarations

Author Contributions

Conceptualization, C.Z., Y.L. and Q.W.; methodology, W.X. and C.G.; formal analysis, W.X. and G.B.; writing—original draft preparation, G.B.; writing—review and editing, G.B., W.X. and C.Z.

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References

1. Zhu, N. et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N. Engl. J Med*382, 727-733 (2020).
2. Rothe, C. et al. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. *N. Engl. J Med*382, 970-971 (2020).
3. Comber, L. et al. Airborne transmission of SARS-CoV-2 via aerosols. *Rev. Med Virol.*31, e2184 (2021).
4. Zou, L. et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *N. Engl. J Med*382, 1177-1179 (2020).
5. Worby, C. J. & Chang, H. H. Face mask use in the general population and optimal resource allocation during the COVID-19 pandemic. *Nat. Commun.*11, 4049 (2020).
6. Borkow, G. Using copper to fight microorganisms. *Curr Chem Biol*6, 93-103 (2012).
7. Behzadinasab, S., Chin, A., Hosseini, M., Poon, L. L. M. & Ducker, W. A. A Surface Coating that Rapidly Inactivates SARS-CoV-2. *ACS. Appl. Mater. Interfaces.*12, 34723-34727 (2020).
8. Borkow, G. & Gabbay, J. Putting copper into action: copper-impregnated products with potent biocidal activities. *FASEB J*18, 1728-1730 (2004).
9. Borkow, G., Salvatori, R. & Kanmukhla, V. K. Drastic Reduction of Bacterial, Fungal and Viral Pathogen Titers by Cuprous Oxide Impregnated Medical Textiles. *J Funct. Biomater.*12, 9 (2021).
10. Borkow, G., Zhou, S. S., Page, T. & Gabbay, J. A novel anti-influenza copper oxide containing respiratory face mask. *PLoS. One.*5, e11295 (2010).
11. Borkow, G. et al. Neutralizing viruses in suspensions by copper oxide based filters. *Antimicrob. Agents Chemother.*51, 2605-2607 (2007).

12. Borkow, G., Lara, H. H., Covington, C. Y., Nyamathi, A. & Gabbay, J. Deactivation of human immunodeficiency virus type 1 in medium by copper oxide-containing filters. *Antimicrob. Agents Chemother.*52, 518-525 (2008).
13. Gabbay, J. et al. Copper oxide impregnated textiles with potent biocidal activities. *Journal of Industrial Textiles*35, 323-335 (2006).
14. Heylings, J. R., Davies, D. J. & Burton, R. Dermal absorption of testosterone in human and pig skin in vitro. *Toxicol. In Vitro.*48, 71-77 (2018).
15. Warnes, S. L., Little, Z. R. & Keevil, C. W. Human Coronavirus 229E Remains Infectious on Common Touch Surface Materials. *mBio.*6, e01697-15 (2015).
16. Doremalen N.V. et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *N. Engl. J Med*382, 1564-1567 (2020).
17. Chin, A. W. H. et al. Stability of SARS-CoV-2 in different environmental conditions. *Lancet Microbe.*1, e10 (2020).
18. Arbosa, M. & Graziano, K. Influence of wearing time on efficacy of disposable surgical masks as microbial barrier. *Br. J. Microbiol*37, 216-217 (2020).
19. Blachere, F. M. et al. Assessment of influenza virus exposure and recovery from contaminated surgical masks and N95 respirators. *J Virol. Methods*260, 98-106 (2018).
20. Chughtai, A. A. et al. Contamination by respiratory viruses on outer surface of medical masks used by hospital healthcare workers. *BMC Infect Dis.*19, 491 (2019).
21. Phan, L. T. et al. Respiratory viruses on personal protective equipment and bodies of healthcare workers. *Infect Control Hosp. Epidemiol.*40, 1356-1360 (2019).
22. Kwok, Y. L., Gralton, J. & McLaws, M. L. Face touching: a frequent habit that has implications for hand hygiene. *Am. J Infect Control*43, 112-114 (2015).
23. Borkow, G., Okon-Levy, N. & Gabbay, J. Copper oxide impregnated wound dressings: biocidal and safety studies. *Wounds*22, 310-316 (2010).
24. Weinberg, I. et al. Safety of using diapers containing copper oxide in chronic care elderly patients. *The Open Biology Journal*6, 54-59 (2013).

Figures

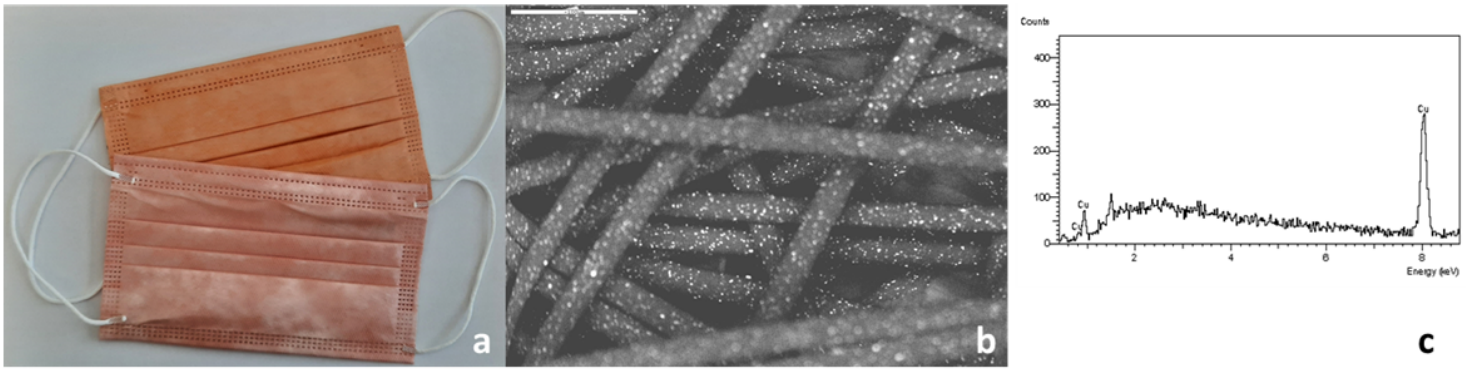

Figure 1

Surgical face masks impregnated with cuprous-oxide microparticles. The external layers of the surgical masks (a) are made from polypropylene spunbond fabric. (b) Scanning electronmicroscopy (SEM)imaging of the polypropylene fabric shows homogenous distribution of white dots on the surface of the polypropylene fibers. (c) A representative Energy Dispersive X-Ray Analysis (EDX) of a white dot seen in (b) shows a peak at 8 keV, corresponding to copper.

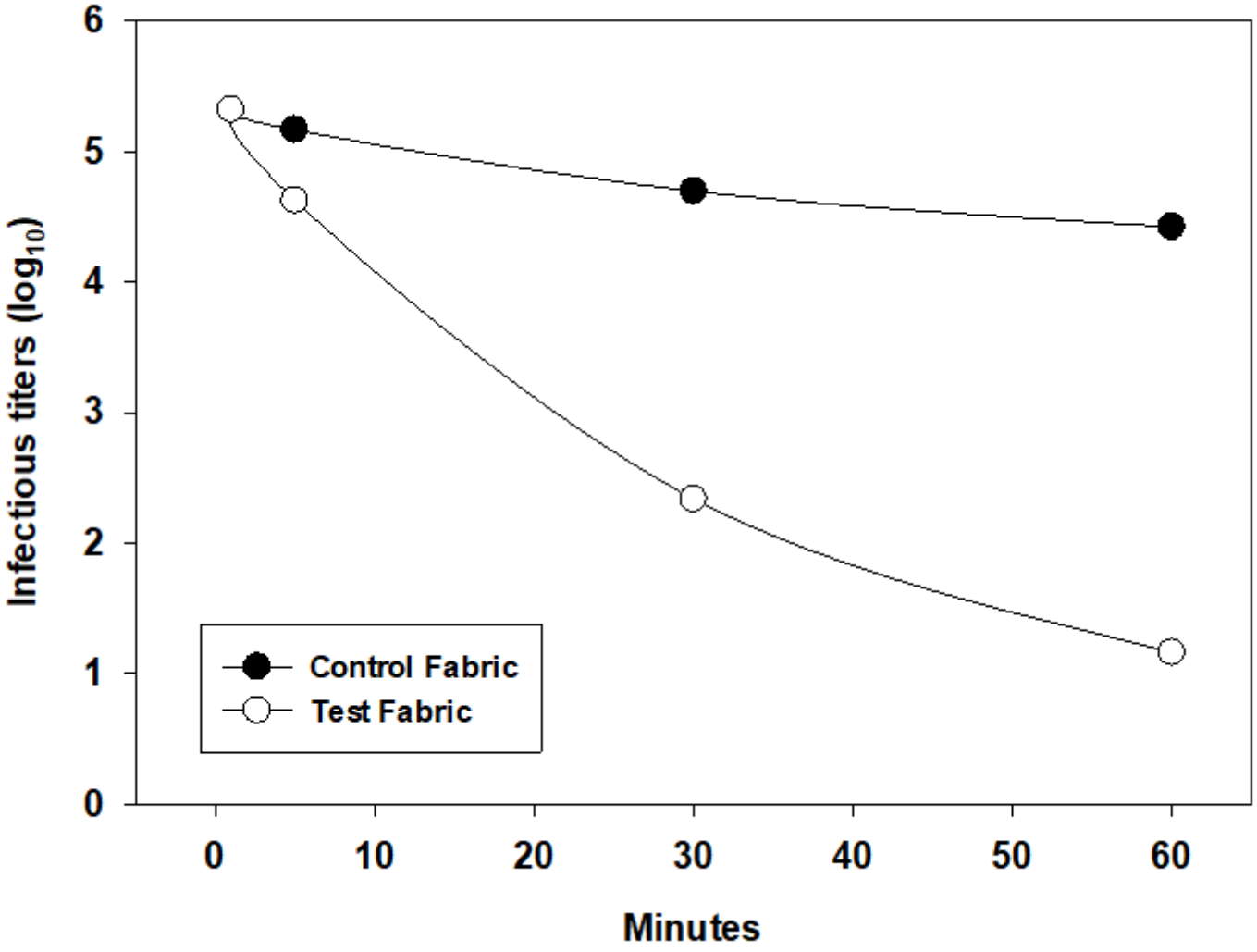

Figure 2

Viral infectious titers of SARS-CoV-2 after being in direct contact with the Control and Test Fabrics. The virions incubated with the fabrics were recovered after the respective incubation times. A plaque assay was then conducted to determine the infectious titers of the recovered virions. The infectious titers of the recovered virus from the Test Fabric were statistically lower than the titers of the virus recovered from the Control Fabric after 5 minutes ($p<0.01$).