Assessment of COVID-19 inactivation effects (virucidal activity) of copper-base Filter material in the reaction of various incubation periods

Request Corps: SK Magic Co., Ltd

Performance Party: College of Veterinary Medicine, Jeonbuk National University, South Korea

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Summary of Test Report

We have assessed the COVID-19 inactivation effects of copper-base filter material supplied by SK magic Corporation using the assays of Cytopathic effects (CPE), Immunofluorescence Examination (IFA), Quantitative Real-Time PCR (qRT-PCR), and Plaque Reduction. The part of the assays was according to the guide lines of ISO18184, and the incubation periods for the reaction were 4hr, 6hr, 18hr, and 20 hr. Based on the results we have confirmed by all the four assays that both copper-base filter materials have the inactivation effects on COVID19 virus and Copper Fiber Ultrafine Dust Filter (CU20) has the better inactivation effect on COVID19 virus compared to Copper Fiber Functional Filter (CU30).

The details of the results are below;

Summary of Test Results

No	Assay	Copper Fiber Ultrafine Dust Filter (CU20)			
		20hr	18hr	6hr	4hr
1	CPE	Inactivated	Inactivated	Inactivated	Inactivated
2	IFA	Not detected	Not detected	Not detected	Not detected
3	qRT-PCR	>99.9%	>99.9%	>99%	>99%
4	Plaque assay	>99.9999%	>99.9999%	>99.999%	>99.999%
No	Assay	Copper Fiber Functional Filter (CU30)			
		20hr	18hr	6hr	4hr
1	CPE	Inactivated	Inactivated	70%	50%
2	IFA	Not detected	Not detected	Not detected	Some detected
3	qRT-PCR	>99.9%	>99.9%	>97%	>97%
4	Plaque assay	>99.9999%	>99.9999%	>99%	>90%

※ These tests were on only the filter based on the assumption under the condition that the virus were captured in/on the filter. Portions of the test methods were followed according to the guide lines of ISO18184.

Contents

Part 1

Assessment of COVID-19 virucidal activity of copper-base Filter material in the reaction of 18 hr and 20hr

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a beta-coronavirus, similar to severe acute respiratory syndrome coronavirus (SARS CoV). The pandemic of COVID-19 is growing, and a shortage of masks and respirators has been reported globally. Prevention of infection with airborne pathogens (viruses, bacteria) can be facilitated through the use of disposable face masks. Development of a biocidal mask (and in general, all protective personal equipment (PPE) capable of rendering the pathogens that come into contact with them non-infectious, may significantly reduce pathogen transmission and contamination of the wearers themselves and the environment. Copper has potent biocidal properties. For example, copper inactivates bacteriophages [1], bronchitis virus [2], poliovirus [3], herpes simplex virus [4], human immunodeficiency virus (HIV), and influenza viruses [5,6]. In the present study, we will evaluate the anti-corona biocidal properties of copper that have incorporated into the fabric of the mask acts as a barrier to the transmission of COVID-19 coronavirus.

Filter material

No	Name	Supplier
1	**Copper Fiber Ultrafine Dust Filter (CU20)**	SK magic Corporation
2	**Copper Fiber Functional Filter (CU30)**	SK magic Corporation

Methodology

Virus and cells

Vero E6 cells were obtained from the American Type Culture Collection (ATCC CRL-1586) and maintained at 37°C with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1× antibiotic-antimycotic solution (Gibco). SARS-CoV-2 (BetaCoV/Korea/KCDC 03/2020) was provided by the Korea Centers for Disease Control and Prevention (KCDC) and was propagated in Vero E6 cells. All experiments using SARS-CoV-2 were performed at Chonbuk National University Zoonotic Center, using enhanced biosafety level 3 (BSL3) containment procedures in laboratories approved for use by the KCDC.

Cytopathic effects (CPE)

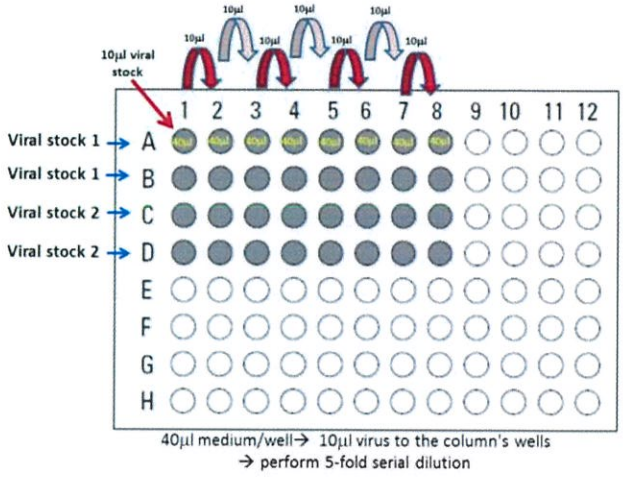
5×10^4 cells/well Vero E6 cells were seeded into 12-well plates in 10% DMEM growth medium and cultured overnight at 37°C in 5% CO_2. Before the experiment filter materials were autoclaved and dried. Then face mask materials will be placed on a new 12 well plate tissue culture plate.

For the viral infections, plates were transferred into the BSL3 containment facility and the filter will be incubated with SARS-CoV2 at different time points (18hr, and 20hr) with a multiplicity of infection (moi) of 0.1, (Pfu/ml = 10,000/ml).

Calculation of infection dose (0.1 moi);

Calculation of the virus titer

1. Seed a 96-well tissue culture plate with Vero E6 cells at 3×10^4 cells/well in 100µl of growth medium.24 hrs later, make a 5-fold serial dilution of viral stock in a round-bottom 96-well plate using serum-free media as shown below.
2. Incubate the plate at 37° C for 2 hrs. After, add 160 µl of growth medium to each well (total 200 µl l/well), continue to incubate the cells at 37°C 5% CO2 incubator.
3. Observe the cells every day to monitor the death of cells.
4. Remove the culture medium from each well. Based on the presence or absence of residual cells, viral titers were calculated in units of log10 50% tissue culture



moi; multiplicity of infection

Virus titer of the original stock: 1×10^{10}/ml

Cell number : 1×10^5 cells/well

Pfu/ml = 0.7× virus titer

= $0.7\times 1.77\times10^{10}$

= 12,390,000,000/ml

moi of original virus stock = $\frac{\text{Pfu/ml}}{\text{Cell number}}$

= $12{,}390{,}000{,}000/2 \times 10^5$

= 61,950 moi

Media of plates containing cells will be removed and washed with 1X PBS meantime after each incubation time, membranes will be washed with 500µl of DMEM and added to the cell culture plates accordingly. Then the cell culture plates will be incubated at 37°C in 5% CO2 incubator for 1 hour.

After 1 hour incubation infection media was removed and 1ml of DMEM containing 2% FBS was added and cultured at 37°C in 5% CO2 incubator for 3 days. After 3rd day of post-infection, the cytopathic effects (CPE) were observed under light microscopy.

Immunofluorescence assay (IFA)

To more intuitively observe the antiviral effect of copper on SARS-CoV2, we performed an immunofluorescence assay. The monolayer of Vero E6 cells infected with 0.1 moi of SARS-CoV2 was exposed to different copper percentages and then fixed in 80% cold acetone for 30 min. Next, the cells were washed 3 times with PBS containing. Then, the primary antibody (SARS-CoV2 S protein, Sino Biological) diluted at a ratio of 1:1000 with 5% BSA was added, and the cells were incubated overnight at 4 °C. The supernatant was discarded, the cells were washed 3 times with PBS, and 1:5000 diluted FITC-labelled Alexa fluor anti-rabbit secondary antibody was added to the cells in the dark and incubated at 37 °C for 1 h. The fluorescence was observed under an inverted Leica fluorescence microscope (Leica, Germany).

Quantitative Real-Time PCR

Virus-containing supernatants were collected from the cells infected with the virus (0.1 moi) with SARS CoV2 at 18h and 20h post-infection. Total RNA was isolated using viral takara Viral DNA/RNA Extraction Kit and used for cDNA synthesis using elpis Reverse transcriptase (elpis Biotech, Daejeon, Korea) according to the manufacturer's instruction. Real-time PCR using applied biosystems (Massachusetts, USA) was performed by subjecting the reaction mixtures to initial denaturation at 94°C for 3 min, followed by 40 cycles of 94°C for 20 sec, 54°C for 20 sec, and 72°C for 30 sec. The primer sequences specific for the Nucleocapsid gene of the SARS CoV2 virus are used for PCR (forward CACATTGGCACCCGCAATC, reverse GAGGAACGAGAAGAGGCTTG).

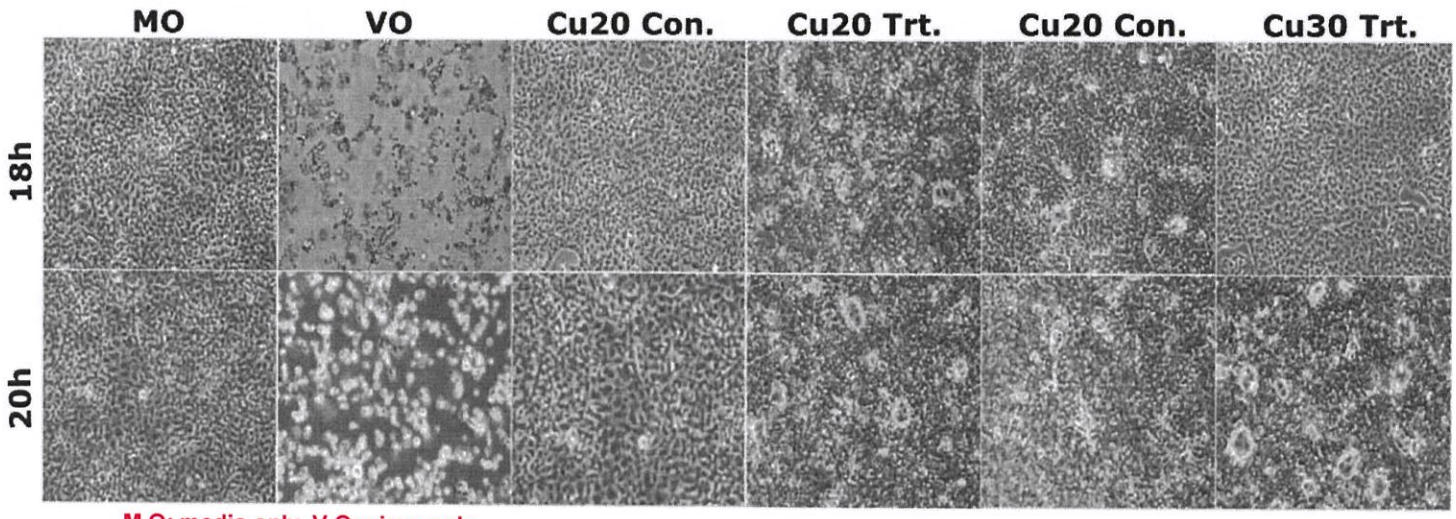
Determination by plaque reduction assay – Assay

Vero E6 cells (1×10^5 per well) were seeded to 24-well plates. Virus incubation with the copper materials will be performed as described above. After each incubation time

virus will be harvested and each sample to be serially diluted by 10-fold (Neg, 10^{-1}, 10^{-2}, 10^{-3}, $10^{-4,}$ 10^{-5}, and 10^{-6}...). Infection and adsorption: Label 24-well plates containing cells (shown below). Remove media and wash cells with 1 ml of PBS. Dispense 100 µl of diluted specimens (Neg, 10^{-1}, 10^{-2}, 10^{-3}, $10^{-4,}$ 10^{-5}, and 10^{-6}...) into designated wells. Incubate at 37°C in a 5% CO_2 incubator for 1hr, rocking every 15 min. Then 2 mL of 0.6% agarose in minimal essential media (MEM) containing 2% FBS and antibiotics was added per well. Plates were incubated at 37°C for 72 hours. The cells were fixed with 10% buffered formalin, followed by the removal of the overlay, and then stained with 0.4% crystal violet to visualize plaque forming units (PFU).

Results

Cytopathic Effect (CPE) Assay

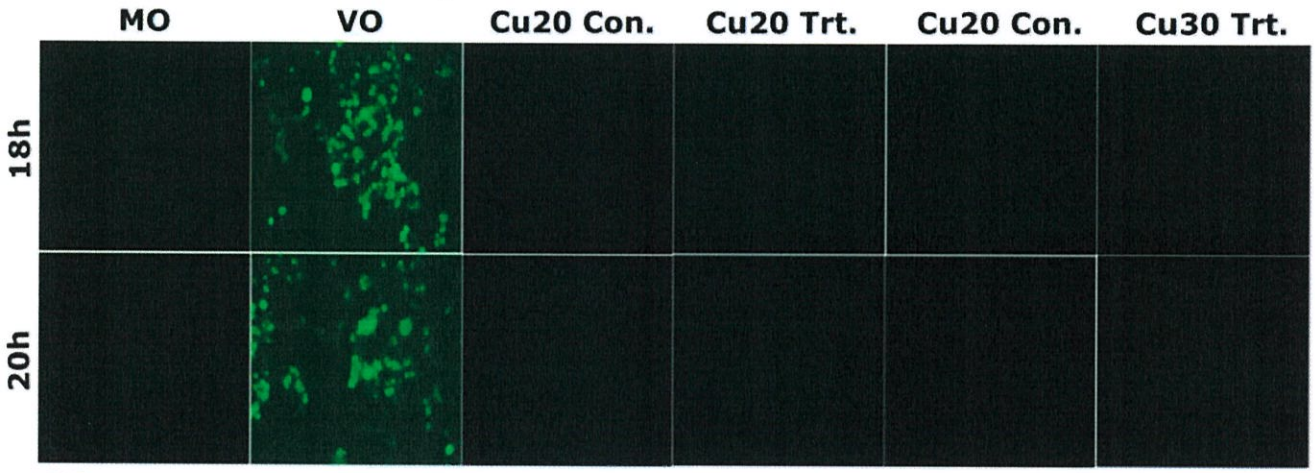


M.O: media only, V.O: virus only

The copper-coated filter materials were screened for their antiviral activities by using CPE inhibition assay. Vero E6 cells were infected at 0.1moi (10,000 pfu/ml) and incubated for 18h, 20h. Non infected VERO E6 cell monolayer after 72 hours, showed no CPE. Filter material with no copper was inactive against SARS-CoV2 at each different infection time points.

Interestingly, after 18hr and 20hr of incubation, the CU20 and CU30 treated and non-treated filter material showed nearly complete inhibitory effect. Whereas virus only group showed complete cytopathic effect.

Immunofluorescence Assay

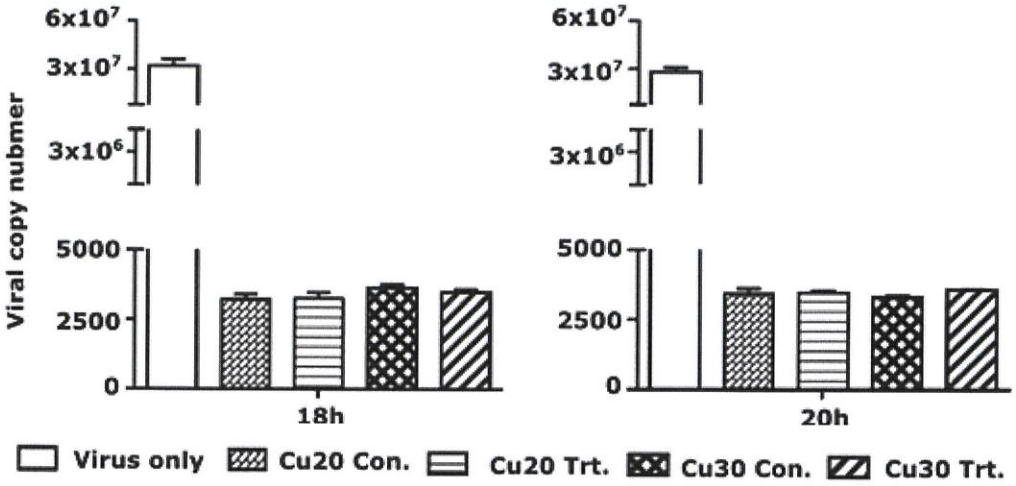


M.O: media only, V.O: virus only

To study the inhibitory impact of copper on the expression of SARS-CoV2 virus S protein on the Vero-E6 cells surface, an indirect immunofluorescence assay (IFA) was performed including both negative and positive controls.

According to the results, a substantial decrease in fluorescence emission intensity in SARS CoV2 incubated with copper-based filters, compared to an intense green fluorescence signal that was observed in no copper group. **After 18h and 20h post incubation there was no fluorescence signal detected from either of CU20 and CU30 treatment and control groups.**

Quantitative real-time RT-PCR (qRT-PCR)



To evaluate the antiviral effects of different copper filters on SARS-CoV2, we examined RNA levels of SARS-CoV2 in Vero E6 cells after incubation SARS-CoV2 on different percentages of copper-based filter materials.

After 18h and 20h of post-incubation the viral copy number was reduced by 99.9% in CU20 and CU30 treated and non-treated filters.

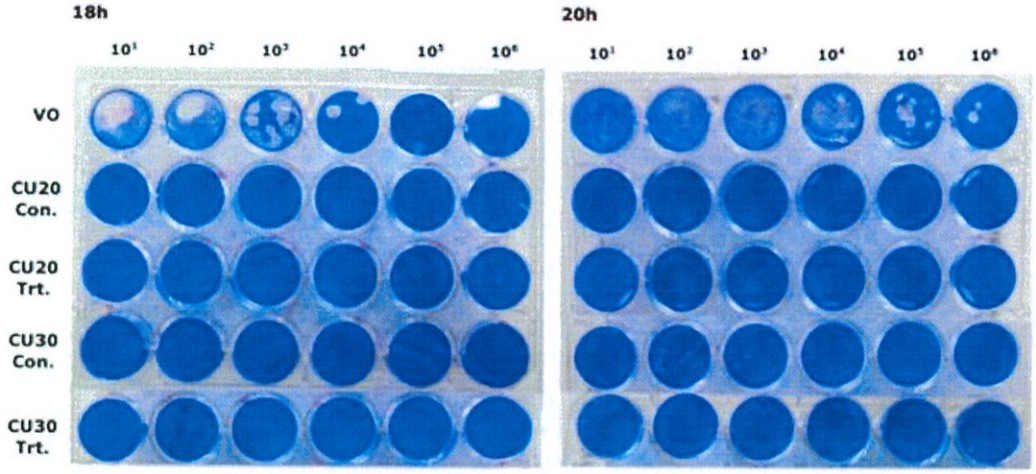
Calculation of fold change and percentage of reduction,

$$\text{Fold} = \frac{\log 10 \text{ of mRNA level in virus only group}}{\log 10 \text{ of mRNA level in the experimental group}}$$

Percentage of reduction =

$$\left(\frac{\log 10 \text{ of mRNA level in virus only group} - \log 10 \text{ of mRNA level in the experimental group}}{\log 10 \text{ of mRNA level in virus only group}}\right) \times 100$$

Plaque reduction assay.



Group	18h		20h	
	Plaque reduction percentage (%)	Log_{10} reduction	Plaque reduction percentage (%)	Log_{10} reduction
CU20 control	100	6.69	100	7
CU20 treatment	100	6.69	100	7
CU30 control	100	6.69	100	7
CU30 treatment	100	6.69	100	7

After incubation for 18h and 20h, SARS-CoV-2 incubated with CU20 and CU30 treated and non-treated filters reduced virus load by 6.69 log_{10} and 7 log_{10} respectively. And showed 100% plaque reduction.

Calculation of plaque reduction percentage,

$$\left(\frac{\text{Number of plaques in the Virus only group} - \text{Number of plaques in the experiment group}}{\text{Number of plaques in the Virus only group}}\right) \times 100$$

Calculation of log_{10} reduction,

$$\frac{\log 10 \text{ of pfu of virus only group} - \log 10 \text{ of pfu of virus only group}}{\log 10 \text{ of pfu of virus only group}}$$

***Pfu=Plaque forming units**

Conclusion

A variety of respiratory pathogenic agents such as influenza, SARS-CoV, MERS-CoV have been exposed to a variety of copper forms in several cultivating media (MDCK, Vero, etc.) having similar results and the same conclusion: Copper is capable to inhibit, inactivate, reduce and irreversibly destroy coronavirus, influenza virus, and other pathogenic agents. A recent study has evaluated and compared SARS-CoV-1 and SARS-CoV-2 stability and decay rates in copper, no viable virus was observed after 8 hours and after 4 hours of SARS-CoV-1 and SARS-CoV-2 respectively.

The described data appears to support the use of copper in mask filters to actively inactivate SARS-CoV2 viruses and it seems to be effective, limiting environmental contamination and a low-cost strategy in reducing transmission of infectious diseases such as the SARS-CoV. It appears that superoxide and hydroxyl radical generation may be important in the inactivation of coronaviruses on copper but that inactivation is primarily due to the direct effect of copper ions. After 18 and 20 hours, the Vero E6 cells infected with viruses which were incubated with copper filters showed cell activity nearly complete viral inhibition, which was similar to that previously reported for the cytotoxicity of soluble copper [7]. After treatment with different concentrations of copper, the viral titer and RNA expression levels of SARS-CoV2 in cells were significantly reduced from those in the control cells.

Interestingly, Copper Fiber Ultrafine Dust Filter (CU20), Copper Fiber functional Filter (Cu30) treated and non-treated filter material had a higher capacity to readily kill the virions that remain in the filter. The reason for this major significance may be due to microbicidal action via the interaction of copper ions with the virions that come into contact with the copper-coated outer surfaces, and it also indicates that the filter material itself also attribute to the antiviral activity.

Part 2

Assessment of COVID-19 virucidal activity of copper-base Filter material in the reaction of 4 hr and 6hr

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a beta-coronavirus, similar to severe acute respiratory syndrome coronavirus (SARS CoV). The pandemic of COVID-19 is growing, and a shortage of masks and respirators has been reported globally. Prevention of infection with airborne pathogens (viruses, bacteria) can be facilitated through the use of disposable face masks. Development of a biocidal mask (and in general, all protective personal equipment (PPE) capable of rendering the pathogens that come into contact with them non-infectious, may significantly reduce pathogen transmission and contamination of the wearers themselves and the environment. Copper has potent biocidal properties. For example, copper inactivates bacteriophages [1], bronchitis virus [2], poliovirus [3], herpes simplex virus [4], human immunodeficiency virus (HIV), and influenza viruses [5,6]. In the present study, we will evaluate the anti-corona biocidal properties of copper that have incorporated into the fabric of the mask acts as a barrier to the transmission of COVID-19 coronavirus.

Filter material

No	Name	Supplier
1	**Copper Fiber Ultrafine Dust Filter (CU20)**	SK magic Corporation
2	**Copper Fiber Functional Filter (CU30)**	SK magic Corporation

Methodology

Virus and cells

Vero E6 cells were obtained from the American Type Culture Collection (ATCC CRL-1586) and maintained at 37°C with 5% CO_2 in Dulbecco's modified Eagle's medium

(DMEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1× antibiotic-antimycotic solution (Gibco). SARS-CoV-2 (BetaCoV/Korea/KCDC 03/2020) was provided by the Korea Centers for Disease Control and Prevention (KCDC) and was propagated in Vero E6 cells. All experiments using SARS-CoV-2 were performed at Chonbuk National University Zoonotic Center, using enhanced biosafety level 3 (BSL3) containment procedures in laboratories approved for use by the KCDC.

Cytopathic effects (CPE)

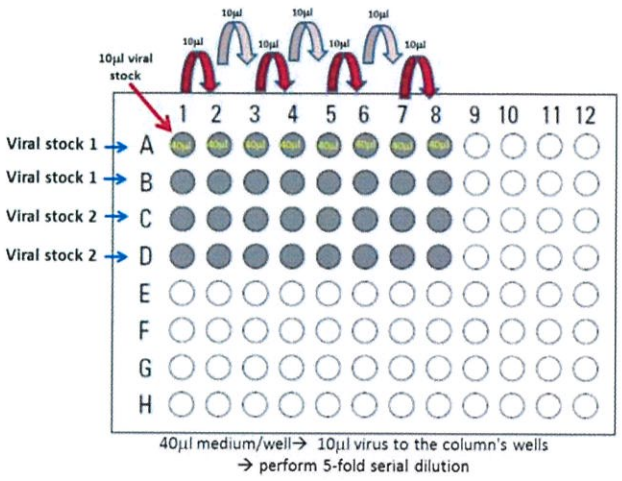
5×10^4 cells/well Vero E6 cells were seeded into 12-well plates in 10% DMEM growth medium and cultured overnight at 37°C in 5% CO_2. Before the experiment filter materials were autoclaved and dried. Then face mask materials will be placed on a new 12 well plate tissue culture plate.

For the viral infections, plates were transferred into the BSL3 containment facility and the filter will be incubated with SARS-CoV2 at different time points (4h, 6h) with a multiplicity of infection (moi) of 0.1, (Pfu/ml = 10,000/ml).

Calculation of infection dose (0.1 moi);

Calculation of the virus titer

1. Seed a 96-well tissue culture plate with Vero E6 cells at 3×10^4 cells/well in 100µl of growth medium 24 hrs later, make a 5-fold serial dilution of viral stock in a round-bottom 96-well plate using serum-free media as shown below.
2. Incubate the plate at 37° C for 2 hrs. After, add 160 µl of growth medium to each well (total 200 µl l/well), continue to incubate the cells at 37°C 5% CO2 incubator.
3. Observe the cells every day to monitor the death of cells.
4. Remove the culture medium from each well. Based on the presence or absence of residual cells, viral titers were calculated in units of $\log^{10}$ 50% tissue culture.



moi; multiplicity of infection

Virus titer of the original stock: 1×10^{10}/ml

Cell number : 1×10^5 cells/well

Pfu/ml = 0.7× virus titer

= $0.7\times 1.77\times10^{10}$

= 12,390,000,000/ml

moi of original virus stock = $\frac{\text{Pfu/ml}}{\text{Cell number}}$

= $12{,}390{,}000{,}000/2 \times 10^5$

= 61,950 moi

Media of plates containing cells will be removed and washed with 1X PBS meantime after each incubation time, membranes will be washed with 500µl of DMEM and added to the cell culture plates accordingly. Then the cell culture plates will be incubated at 37°C in 5% CO2 incubator for 1 hour.

After 1 hour incubation infection media was removed and 1ml of DMEM containing 2% FBS was added and cultured at 37°C in 5% CO2 incubator for 3 days. After 3rd day of post-infection, the cytopathic effects (CPE) were observed under light microscopy.

Immunofluorescence assay (IFA)

To more intuitively observe the antiviral effect of copper on SARS-CoV2, we performed an immunofluorescence assay. The monolayer of Vero E6 cells infected with 0.1 moi of SARS-CoV2 was exposed to different copper percentages and then fixed in 80% cold acetone for 30 min. Next, the cells were washed 3 times with PBS containing. Then, the primary antibody (SARS-CoV2 S protein, Sino Biological) diluted at a ratio of 1:1000 with 5% BSA was added, and the cells were incubated overnight at 4 °C. The supernatant was discarded, the cells were washed 3 times with PBS, and 1:5000 diluted FITC-labelled Alexa fluor anti-rabbit secondary antibody was added to the cells in the dark and incubated at 37 °C for 1 h. The fluorescence was observed under an inverted Leica fluorescence microscope (Leica, Germany).

Quantitative Real-Time PCR

Virus-containing supernatants were collected from the cells infected with the virus (0.1 moi) with SARS CoV2 at 4h, 6h post-infection. Total RNA was isolated using viral takara Viral DNA/RNA Extraction Kit and used for cDNA synthesis using elpis Reverse transcriptase (elpis Biotech, Daejeon, Korea) according to the manufacturer's instruction. Real-time PCR using applied biosystems (Massachusetts, USA) was performed by subjecting the reaction mixtures to initial denaturation at 94°C for 3 min, followed by 40 cycles of 94°C for 20 sec, 54°C for 20 sec, and 72°C for 30 sec. The primer sequences specific for the Nucleocapsid gene of the SARS CoV2 virus are used for PCR (forward CACATTGGCACCCGCAATC, reverse GAGGAACGAGAAGAGGCTTG).

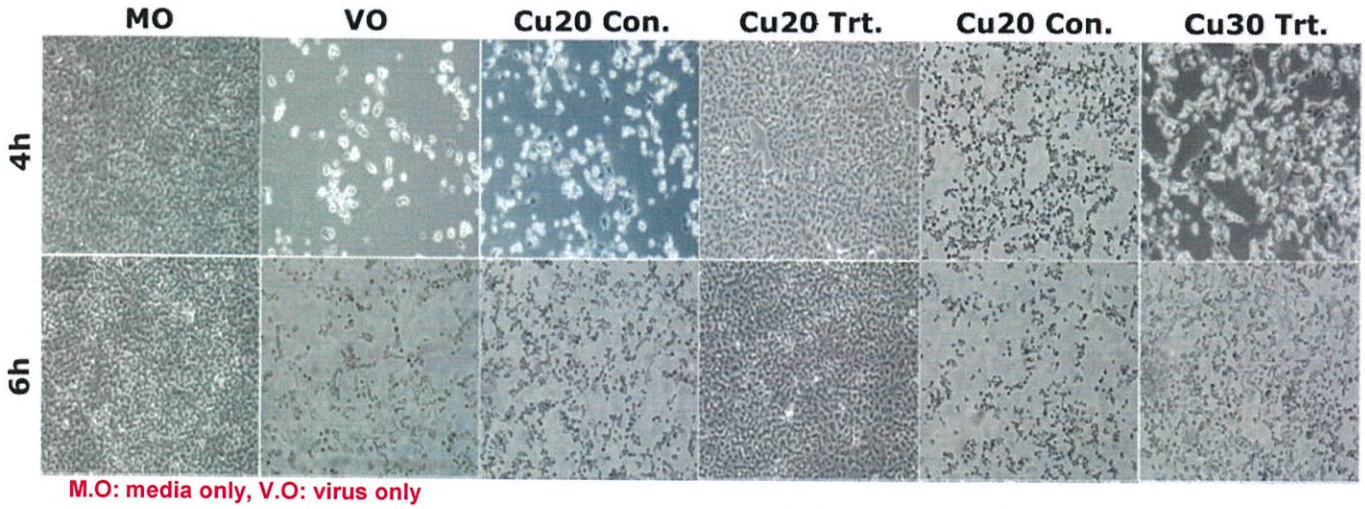
Determination by plaque reduction assay – Assay

Vero E6 cells (1×10^5 per well) were seeded to 24-well plates. Virus incubation with the copper materials will be performed as described above. After each incubation time

virus will be harvested and each sample to be serially diluted by 10-fold (Neg, 10^{-1}, 10^{-2}, 10^{-3}, $10^{-4,}$ 10^{-5}, and 10^{-6}...). Infection and adsorption: Label 24-well plates containing cells (shown below). Remove media and wash cells with 1 ml of PBS. Dispense 100 µl of diluted specimens (Neg, 10^{-1}, 10^{-2}, 10^{-3}, $10^{-4,}$ 10^{-5}, and 10^{-6}...) into designated wells. Incubate at 37°C in a 5% CO_2 incubator for 1hr, rocking every 15 min. Then 2 mL of 0.6% agarose in minimal essential media (MEM) containing 2% FBS and antibiotics was added per well. Plates were incubated at 37°C for 72 hours. The cells were fixed with 10% buffered formalin, followed by the removal of the overlay, and then stained with 0.4% crystal violet to visualize plaque forming units (PFU).

Results

Cytopathic Effect (CPE) Assay



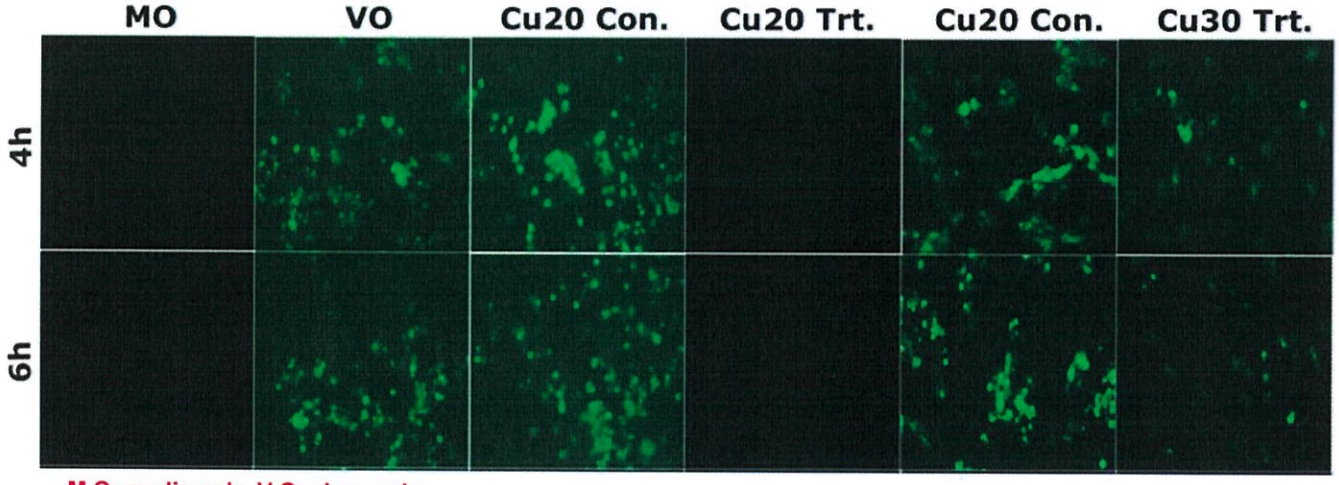
M.O: media only, V.O: virus only

The copper-coated filter materials were screened for their antiviral activities by using CPE inhibition assay. Vero E6 cells were infected at 0.1moi (10,000 pfu/ml) and incubated for 4h, 6h. As shown in the figure, filter materials coated with copper showed significant inhibition of the cytopathic effect induced by the SARS-CoV2 after 4 hour post-incubation.

Non infected VERO E6 cell monolayer after 72 hours, showed no CPE. Filter material with no copper was inactive against SARS-CoV2 at each different infection time points.

After 4hr and 6hr of incubation, the CU20 filter material showed nearly complete inhibitory effect. Whereas Cu30 filter material showed near 50% and 70% CPE inhibitory affect after 4h and 6h co-incubation respectively.

Immunofluorescence Assay



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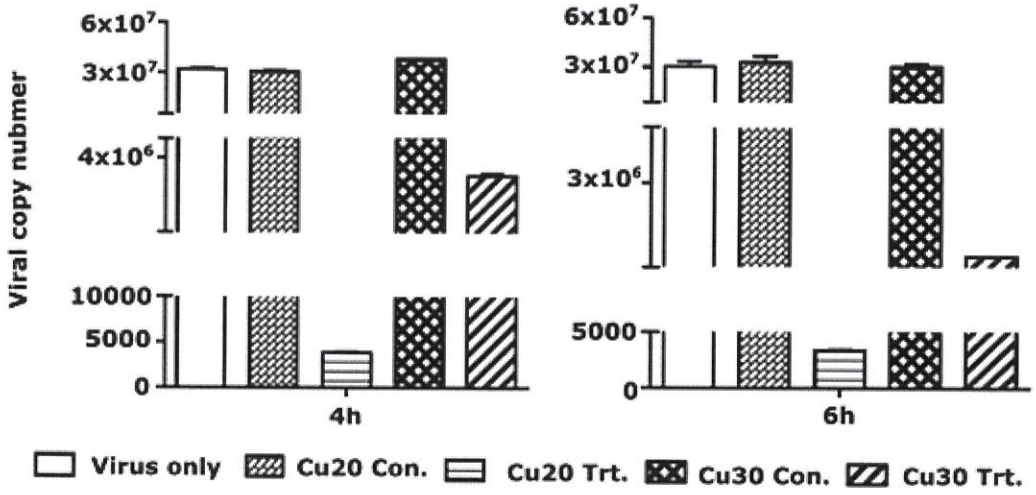
To study the inhibitory impact of copper on the expression of SARS-CoV2 virus S protein on the Vero-E6 cells surface, an indirect immunofluorescence assay (IFA) was performed including both negative and positive controls.

According to the results, a substantial decrease in fluorescence emission intensity in SARS CoV2 incubated with copper-based filters, compared to an intense green fluorescence signal that was observed in no copper group. **After 4 and 6 hours of incubation, there was no fluorescence signal at cells treated with Cu20-base filter materials to that of the control group. Whereas CU30 showed slightly higher fluorescence intensity to CU20 but less than the control groups.**

Quantitative real-time RT-PCR (qRT-PCR)



To evaluate the antiviral effects of different copper filters on SARS-CoV2, we examined RNA levels of SARS-CoV2 in Vero E6 cells after incubation SARS-CoV2 on different percentages of copper-based filter materials.

After 4h and 6h of infection, in the presence of Cu20 filter, the SARS-CoV2 RNA replication levels were reduced by 99%% at each time points. For Cu30 filter, after 4h and 6h incubation viral copy number was reduced by 97%.

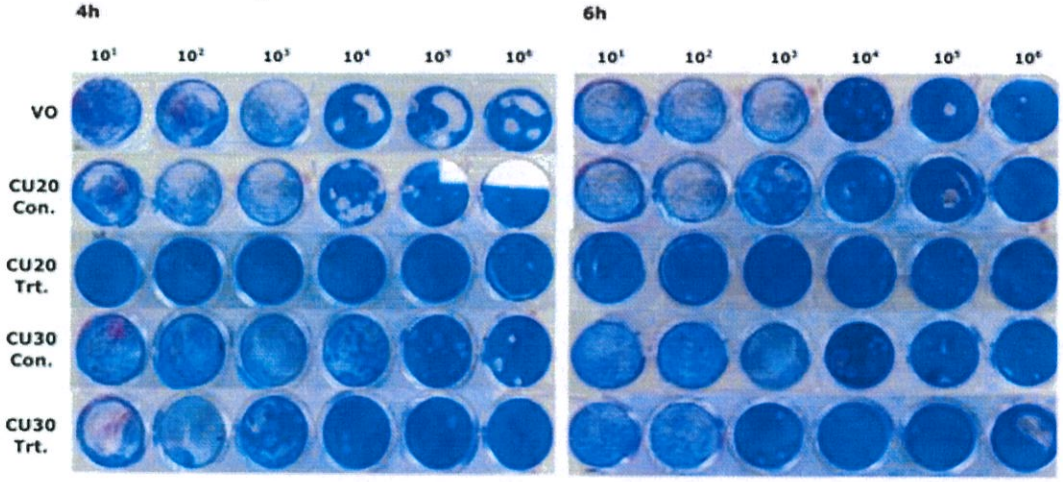
Calculation of fold change and percentage of reduction,

$$\text{Fold} = \frac{\log 10 \text{ of mRNA level in virus only group}}{\log 10 \text{ of mRNA level in the experimental group}}$$

Percentage of reduction =

$$\left(\frac{\log 10 \text{ of mRNA level in virus only group} - \log 10 \text{ of mRNA level in the experimental group}}{\log 10 \text{ of mRNA level in virus only group}}\right) \times 100$$

Plaque reduction assay.



Group	4h		6h	
	Plaque reduction percentage (%)	Log_{10} reduction	Plaque reduction percentage (%)	Log_{10} reduction
CU20 control		0.3		0.34
CU20 treatment		6.3		5.95
CU30 control		0.12		0.22
CU30 treatment		1.47		2.65

After 4 and 6 hour post incubation virus incubated with CU20 filter reduced virus load by 6.3 log_{10} and 5.95 log_{10}, respectively. Whereas CU30 reduced virus load by 1.47 log_{10} and 2.6 log_{10}, respectively.

Calculation of plaque reduction percentage,

$$\left(\frac{\text{Number of plaques in the Virus only group} - \text{Number of plaques in the experiment group}}{\text{Number of plaques in the Virus only group}}\right) \times 100$$

Calculation of log_{10} reduction,

$$\frac{\log 10 \text{ of pfu of virus only group} - \log 10 \text{ of pfu ofthe experimental group}}{\log 10 \text{ of pfu of virus only group}}$$

***Pfu=Plaque forming units**

Conclusion

A variety of respiratory pathogenic agents such as influenza, SARS-CoV, MERS-CoV have been exposed to a variety of copper forms in several cultivating media (MDCK, Vero, etc.) having similar results and the same conclusion: Copper is capable to inhibit, inactivate, reduce and irreversibly destroy coronavirus, influenza virus, and other pathogenic agents. A recent study has evaluated and compared SARS-CoV-1 and SARS-CoV-2 stability and decay rates in copper, no viable virus was observed after 8 hours and after 4 hours of SARS-CoV-1 and SARS-CoV-2, respectively.

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Interestingly, Copper Fiber Ultrafine Dust Filter (CU20) had a higher capacity to readily kill the virions that remain in the filter. The reason for this major significance may be due to microbicidal action via the interaction of copper ions with the virions that come into contact with the copper-coated outer surfaces.

References

[1] Yahya MT, Gerba CP, Straub TM, Margolin AB. Inactivation of bacteriophage MS-2 and poliovirus in copper, galvanized and plastic domestic water pipes. Int J Environ Health Res 1991. doi:10.1080/09603129109356707.

[2] Jordan FT, Nassar TJ. The influence of copper on the survival of infectious bronchitis vaccine virus in water. Vet Rec 1971. doi:10.1136/vr.89.23.609.

[3] Sagripanti JL, Routson LB, Lytle CD. Virus inactivation by copper or iron ions alone and in the presence of peroxide. Appl Environ Microbiol 1993. doi:10.1128/aem.59.12.4374-4376.1993.

[4] Sagripanti JL, Routson LB, Bonifacino AC, Lytle CD. Mechanism of copper-mediated inactivation of herpes simplex virus. Antimicrob Agents Chemother 1997. doi:10.1128/aac.41.4.812.

[5] Sagripanti JL, Lightfoote MM. Cupric and ferric ions inactivate HIV. AIDS Res Hum Retroviruses 1996. doi:10.1089/aid.1996.12.333.

[6] Horie M, Ogawa H, Yoshida Y, Yamada K, Hara A, Ozawa K, et al. Inactivation and morphological changes of avian influenza virus by copper ions. Arch Virol 2008. doi:10.1007/s00705-008-0154-2.

[7] Borkow G, Zhou SS, Page T, Gabbay J. A novel anti-influenza copper oxide containing respiratory face mask. PLoS One 2010. doi:10.1371/journal.pone.0011295.