ASSESSMENT OF COVID-19 VIRUS VIRUCIDAL ACTIVITY OF COPPER-BASED 3D TEXTURED RESPIRATORY FACE MASK OF LSK FINETEX

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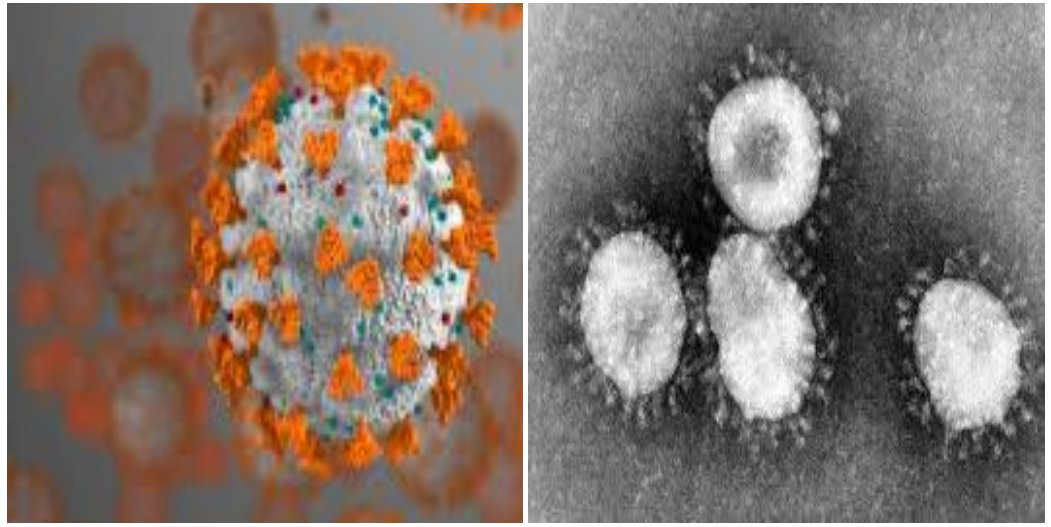
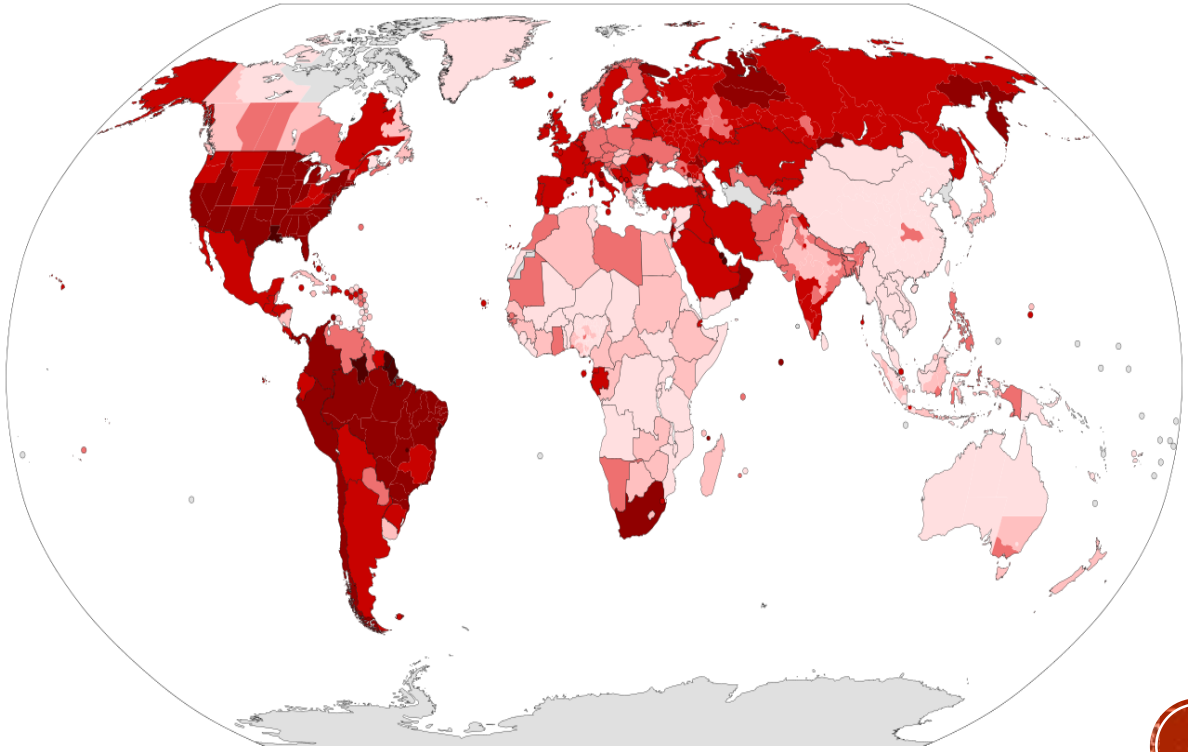
CONTENT

- Background
- Materials and Methods
- Results
- Conclusion

BACKGROUND

- Coronavirus disease 2019 (COVID-19) is caused by the novel beta-coronavirus family member coined SARS-CoV-2.
- In December 2019, a cluster of patients with pneumonia of unknown etiology were identified in Wuhan, China.
- The World Health Organization (WHO) declared COVID-19 a pandemic.
- In the Republic of Korea, as of August 24, 2020, 17,665 confirmed cases leading to 309 deaths have occurred.





BACKGROUND

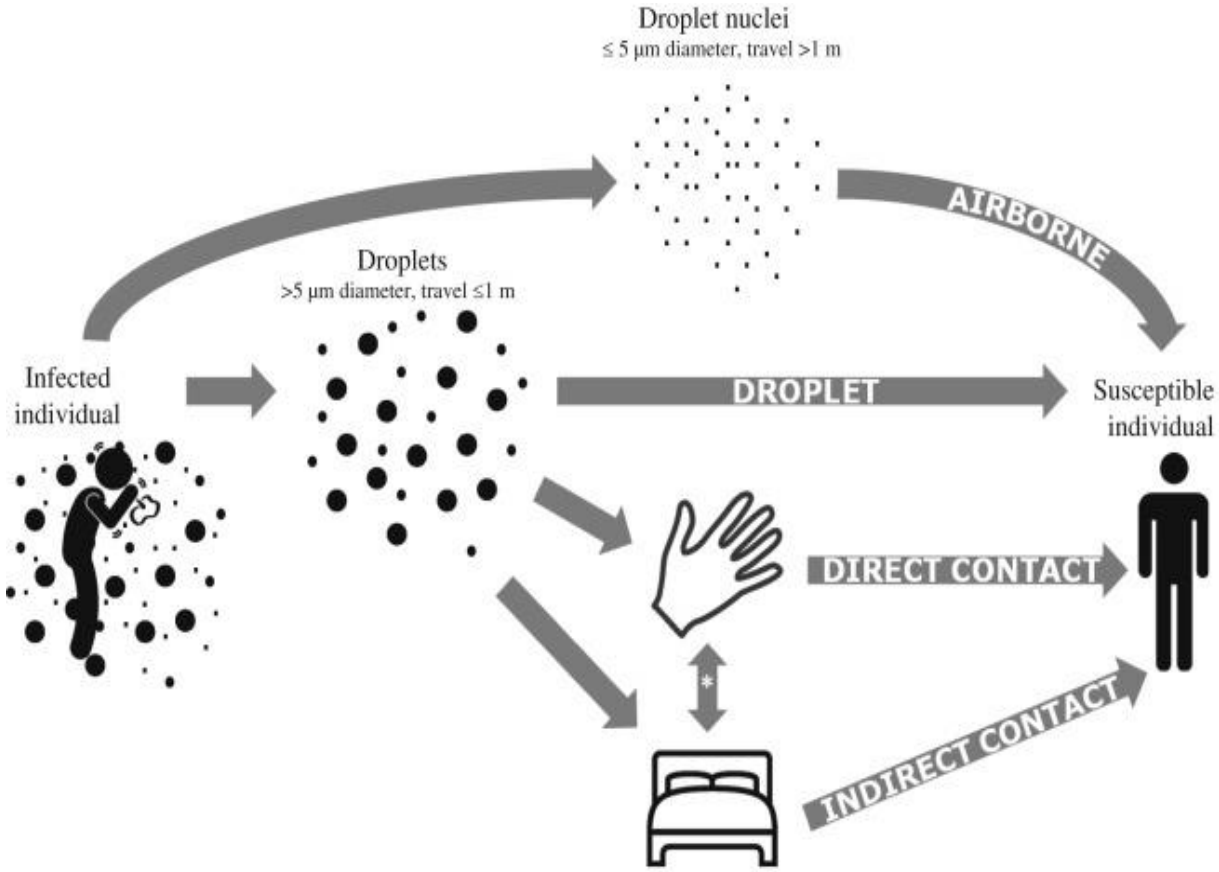
- Transmission of SARS-CoV2

Primary mechanism of transmission is direct and indirect droplet spread.

Direct droplet spread is occur when respiratory particles greater than 5 μm in diameter make contact with the mucosal surface of a recipient.

Indirect spread occurs when a fomite or an intermediate surface is touched, which then contacts mucosal surfaces.

Additional studies are urgently needed to curb the spread of viral infections.

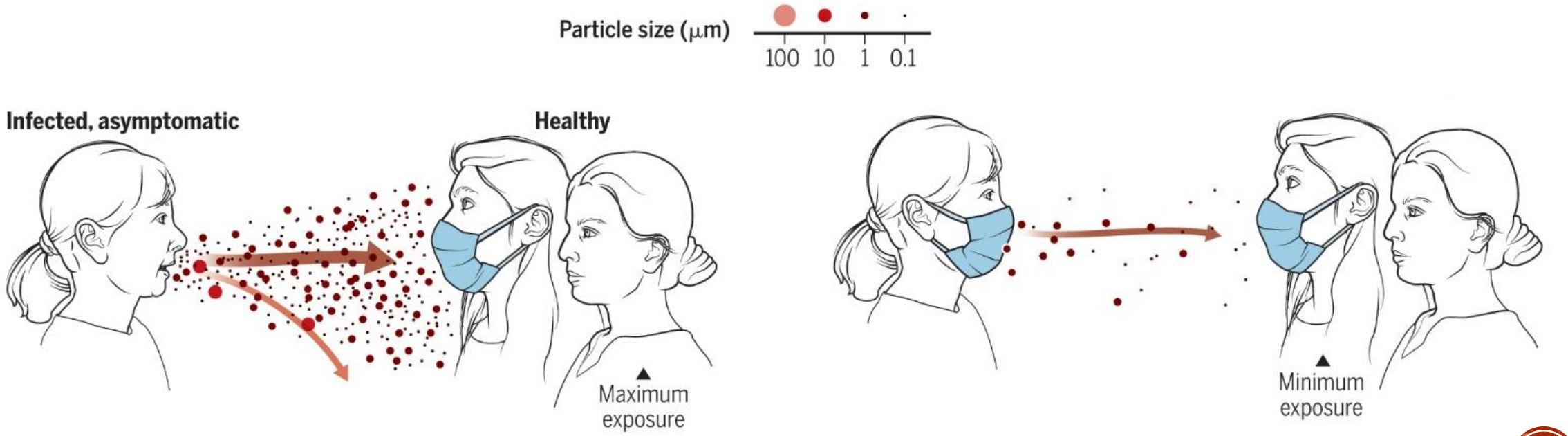


BACKGROUND

- Considerations for Wearing Masks- Help Slow the Spread of COVID-19

The use of respiratory protection devices has been shown to be an effective non-pharmaceutical intervention to reduce the spread of respiratory viruses

Masks are especially important in enclosed spaces and should be worn in all community settings.



BACKGROUND

- **Copper based face mask-to protect against Coronavirus**

Most of the commercial masks are unable to sufficiently filter the tiny aerosols containing the viruses.

Viruses which are attached to the filtering materials can penetrate through the moist mask, and increase the risk of infection.

Improving the antiviral capability of the face masks, can reduce the risk of cross-infection or secondary infection during the usage or handling.

Due to the potent antiviral and antibacterial properties of copper, it is believed that these masks confer protection from pathogens.

BACKGROUND

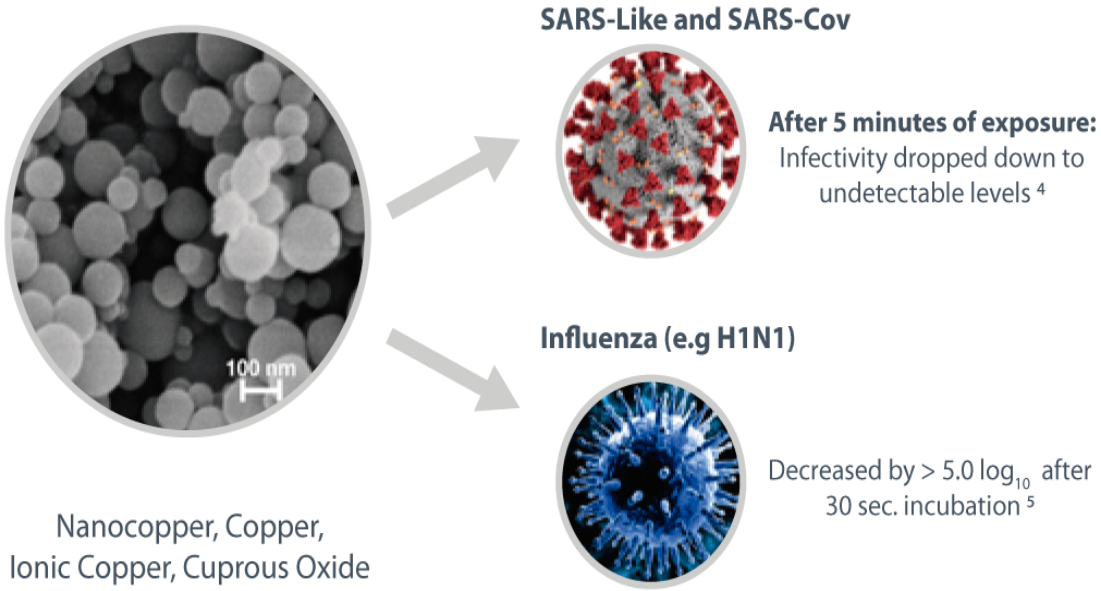
- **Antiviral properties of copper**

Copper is considered safe to humans as due to its low sensitivity of human tissue.

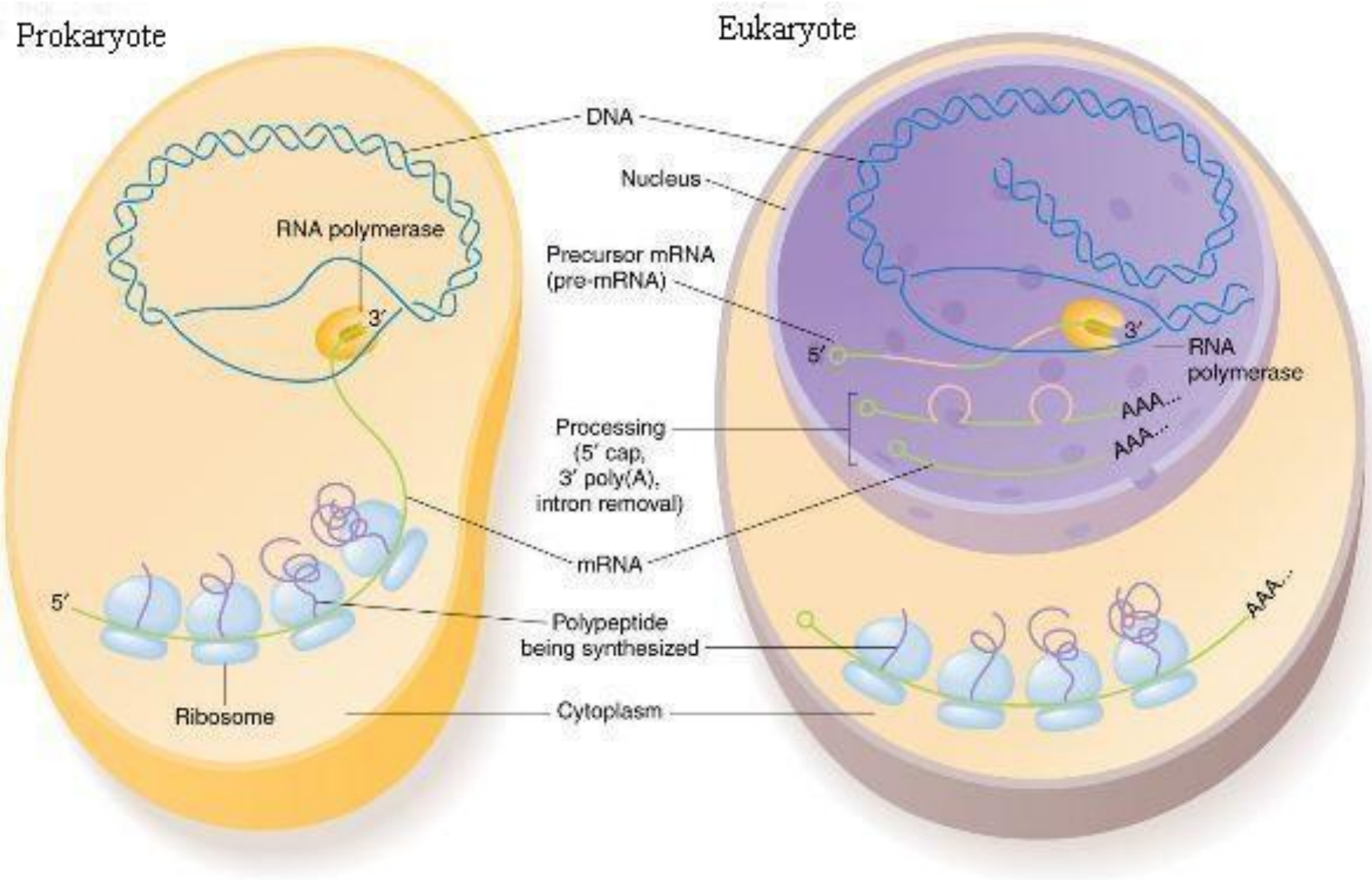
It appears that the antimicrobial activity of copper is directly linked to the oxidative behavior of copper.

The metal virucidal effect was enhanced by the addition of peroxide, particularly for Cu2+.

In every case, the viruses were more resistant to iron-peroxidase than copper-peroxidase on a metal concentration basis.



STRUCTURAL DIFFERENCES BETWEEN EUKARYOTIC(ANIMAL AND HUMAN) AND PROKARYOTIC (BACTERIA AND VIRUS)CELLS



BACKGROUND

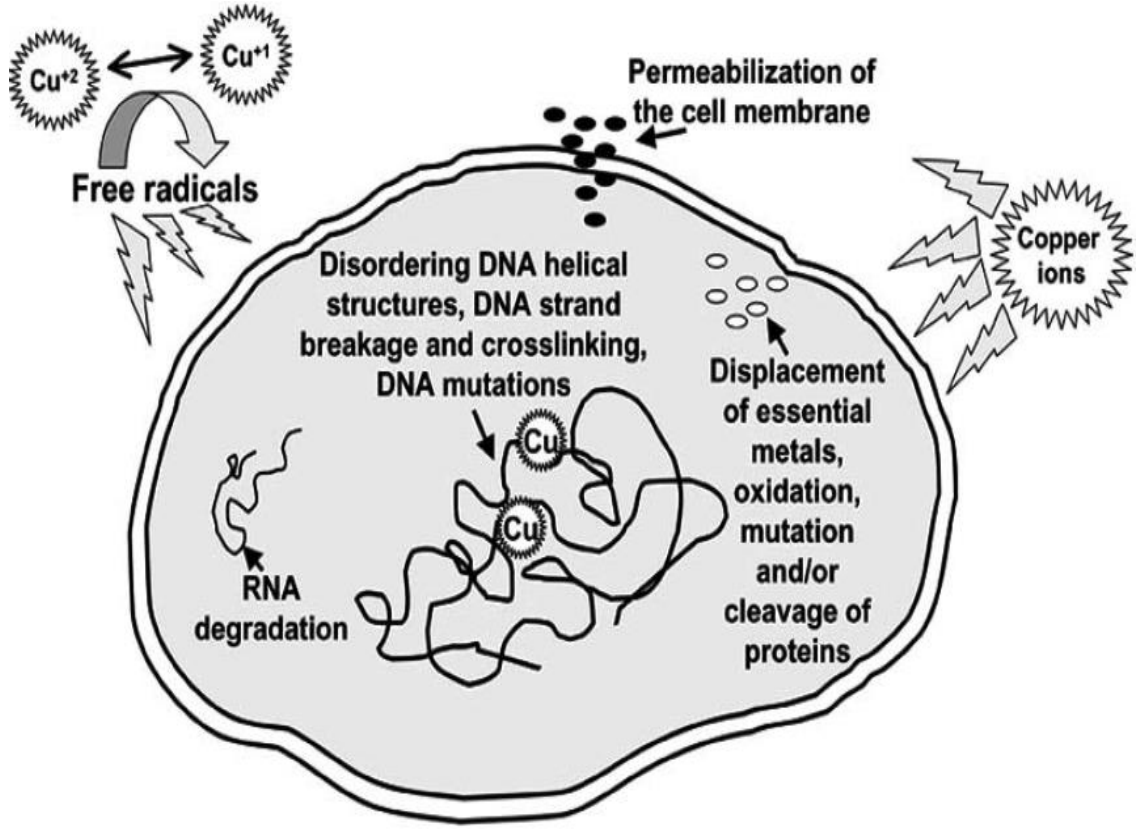
- **Mechanisms of Toxicity of Copper to Microorganisms**

Cu2+ has a specific affinity for DNA and can bind and disorder helical structures by crosslinking within and between strands.

Cu2+ reversibly denatures DNA in low ionic strength solutions competing with hydrogen bonding present within the DNA molecule.

Cu2+ also cause loss of the permeability barrier of the plasma membrane lead to a rapid decline in membrane integrity.

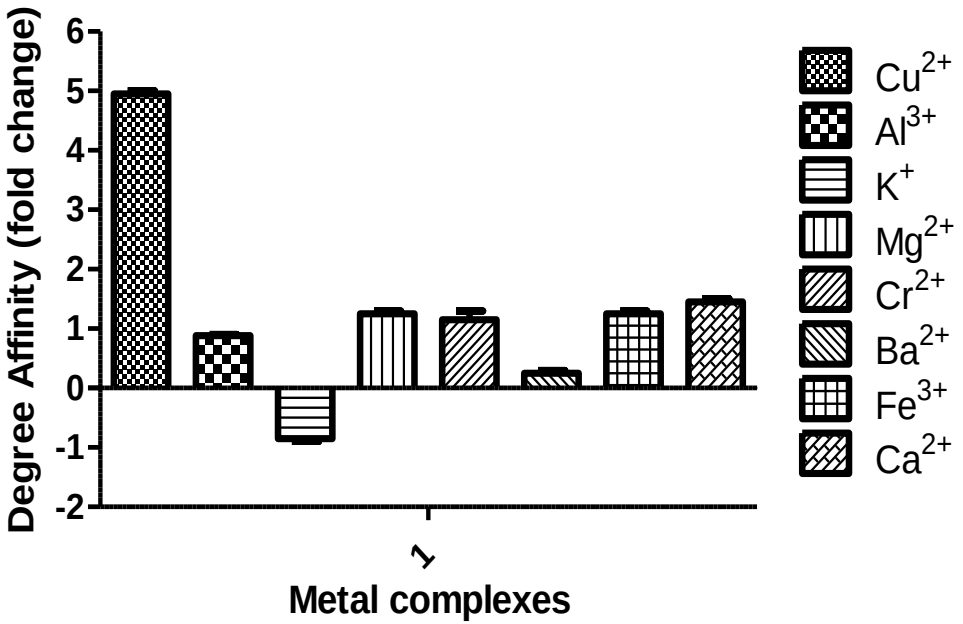
Since, viruses do not possess resistance or repair mechanisms, that makes them highly susceptible to high concentrations of copper ions.

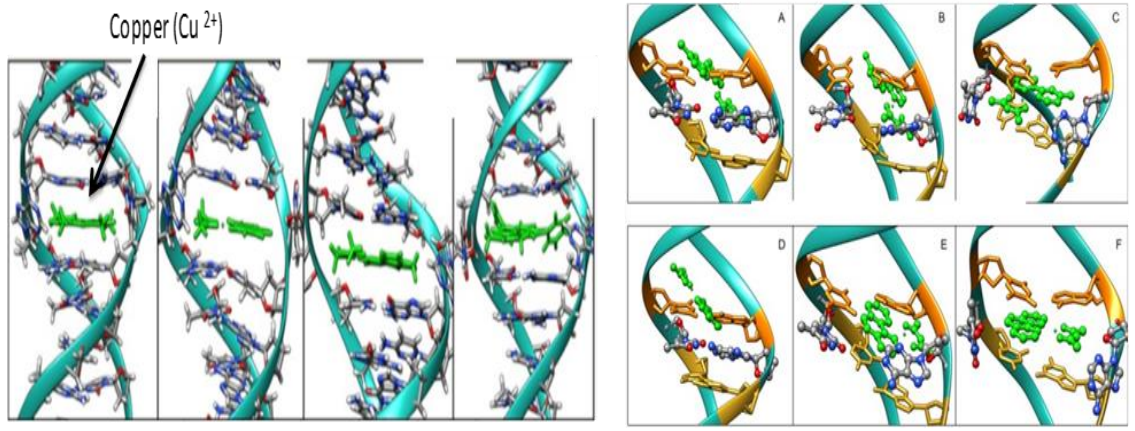


BACKGROUND

- **Mechanisms of Toxicity of Copper to Microorganisms cont.**

Among the various metal ions ($Fe3^{+}$, Cu^{2+}, Zn^{2+}, and Cd^{2} etc.) examined for their inactivation effect on protein tyrosine phosphatase and DNA, Cu^{2+} was found to be the most potent in-activator.





Inter and intramolecular interaction between opposite strands of DNA and bases & sugar within the same DNA/RNA leads in disruption of hydrogen and phosphate bonds respectively.

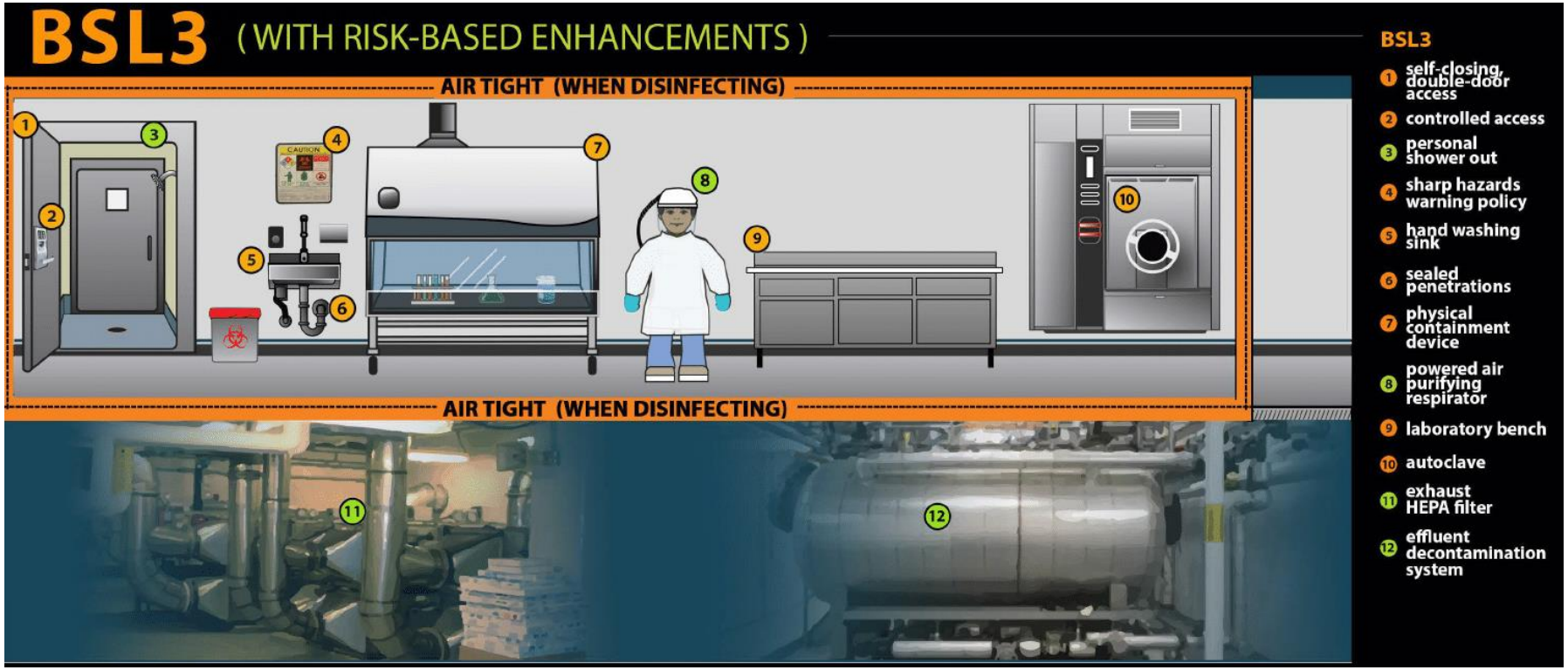
green-copper complex, cyan- backbone

MATERIALS AND METHODS

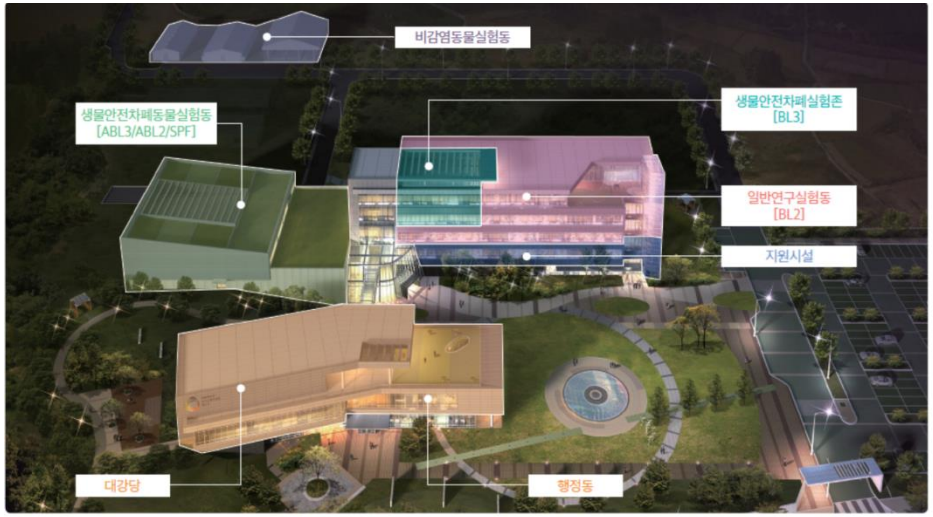
- **Virus and cells**

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 Jeonbuk National University Zoonotic Center, using enhanced biosafety level 3 (BSL3) containment procedures in laboratories approved for use by the KCDC.



Korea Zoonosis Research Institute, Jeonbuk National University




ABL3 특수생물안전차폐동물실험실


BL3 특수생물안전차폐실험실


대동물실험실(돼지·개)


중동물실험실(가금)


소동물실험실(마우스·랫트)


대형사체처리탱크


BL2 생물안전실험실

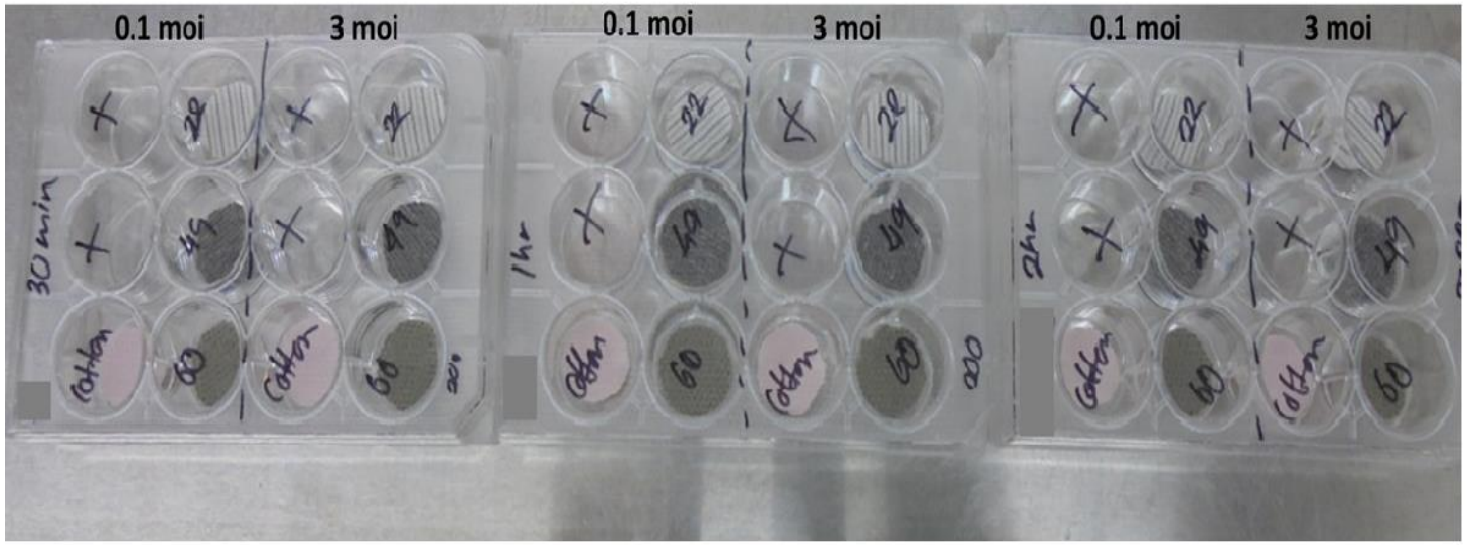

중동물실험실(토끼·페렛)

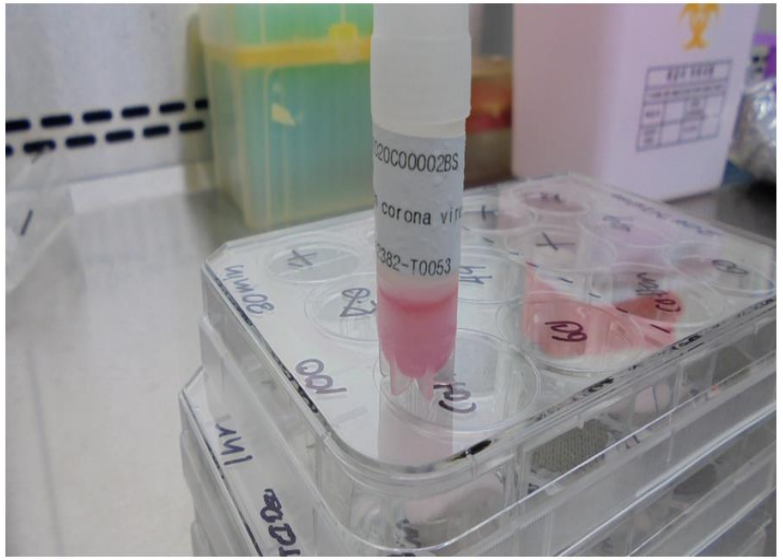
MATERIALS AND METHODS

- **Cytopathic effects (CPE)**

5 × 10^4 cells/well Vero E6 cells were seeded into 12-well plates in DMEM growth medium and cultured overnight at 37°C in 5% CO_2.

Before the experiment mask materials were autoclaved and dried. Then face mask materials will be placed on a new 12 well plate tissue culture plates.





For the viral infections, membranes will be incubated with SARS-CoV2 at different time points (30min, 1hr, and 2hr) with a multiplicity of infection (moi) of 0.1.

MATERIALS AND METHODS

- **Cytopathic effects (CPE) cont.**

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.

MATERIALS AND METHODS

- **Immunofluorescence assay (IFA)**

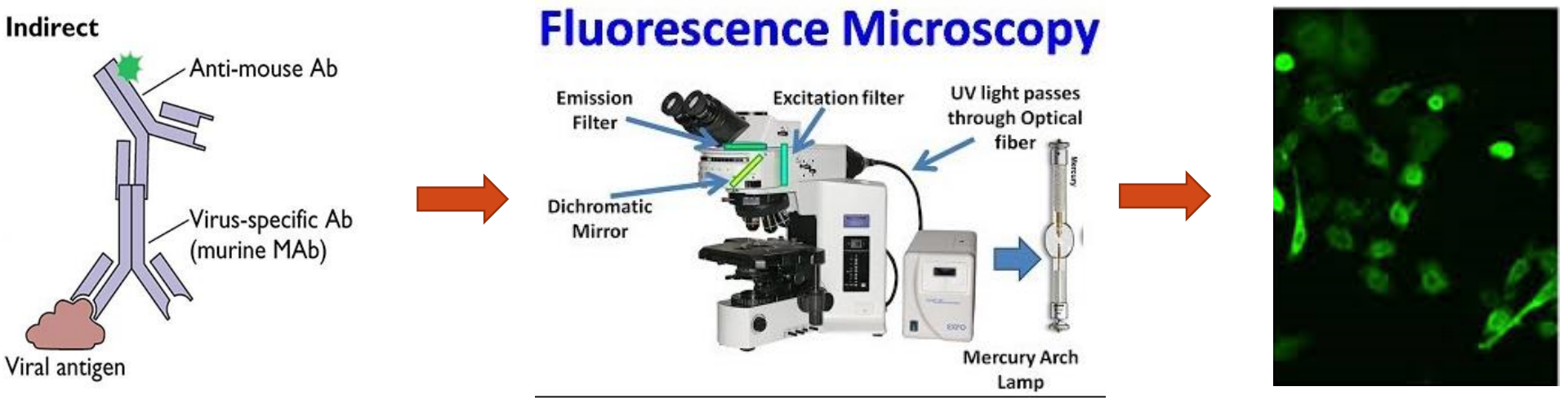
To more intuitively observe the antiviral effect of copper on SARS-CoV2, we performed an immunofluorescence assay.

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.

Cells were incubated with, the primary antibody (SARS-CoV2 S protein) and incubated overnight at 4 °C.

Cells were incubated with FITC-labelled secondary antibody at 37 °C for 1 h.

The fluorescence was observed under an inverted Leica fluorescence microscope.



MATERIALS AND METHODS

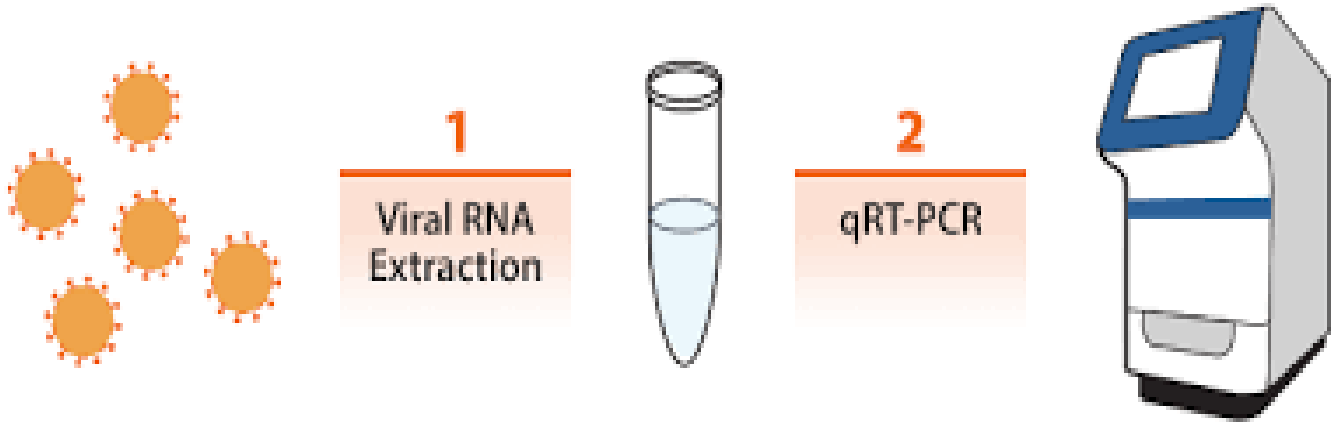
- **Quantitative Real-Time PCR**

Virus-containing supernatants were collected from the cells infected with SARS CoV2 at 30min, 1 hour, and 2 hours post-infection.

Total RNA was isolated and cDNA was synthesized.

Real-time PCR 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.

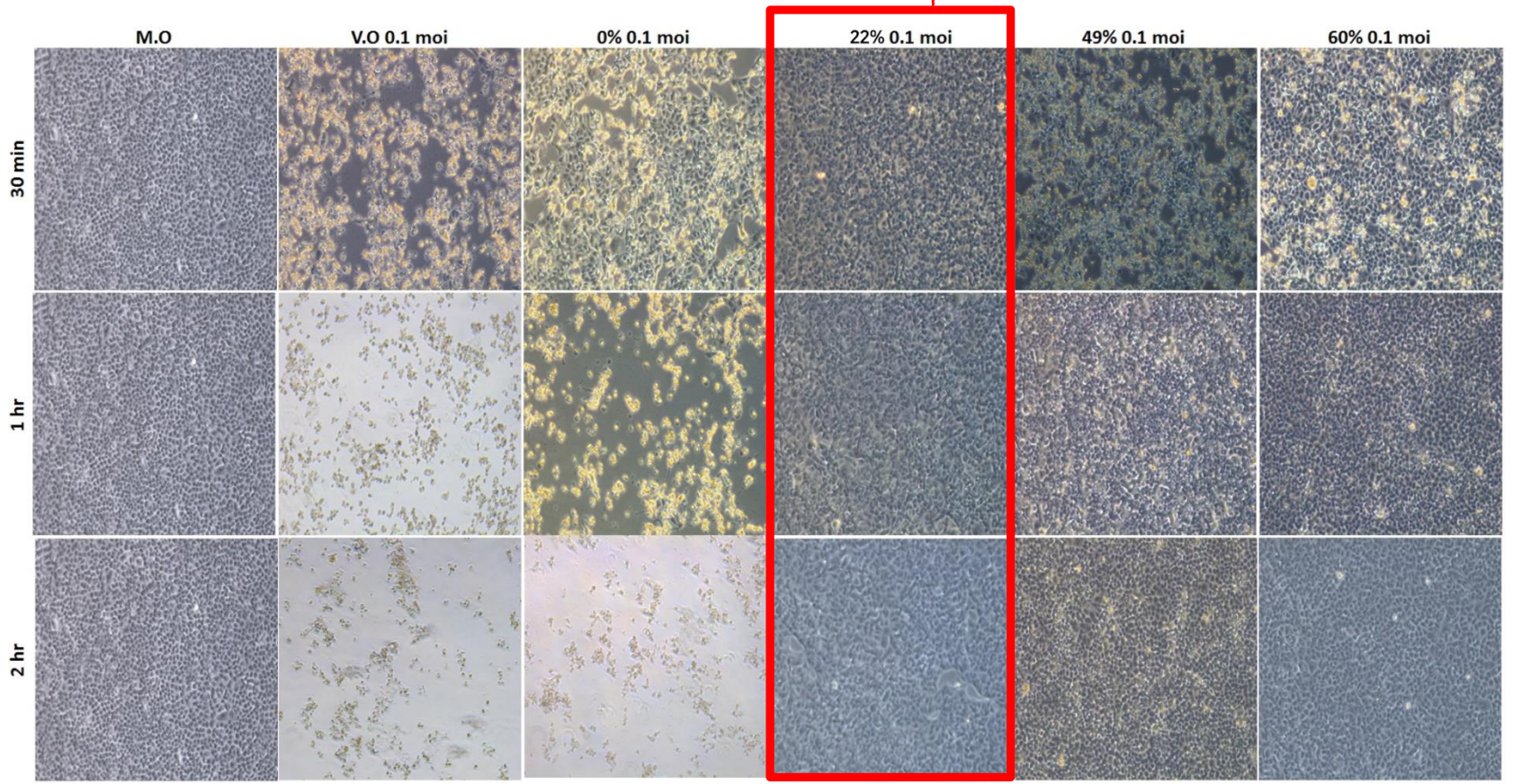
The primer sequences specific for the Nucleocapsid gene of the SARS CoV2 virus are used for PCR (forward CACATTGGCACCCGCAATC, reverse GAGGAACGAGAAGAGGCTTG).



RESULTS

3D textured with Copperline mask

- **Cytopathic effect assay**



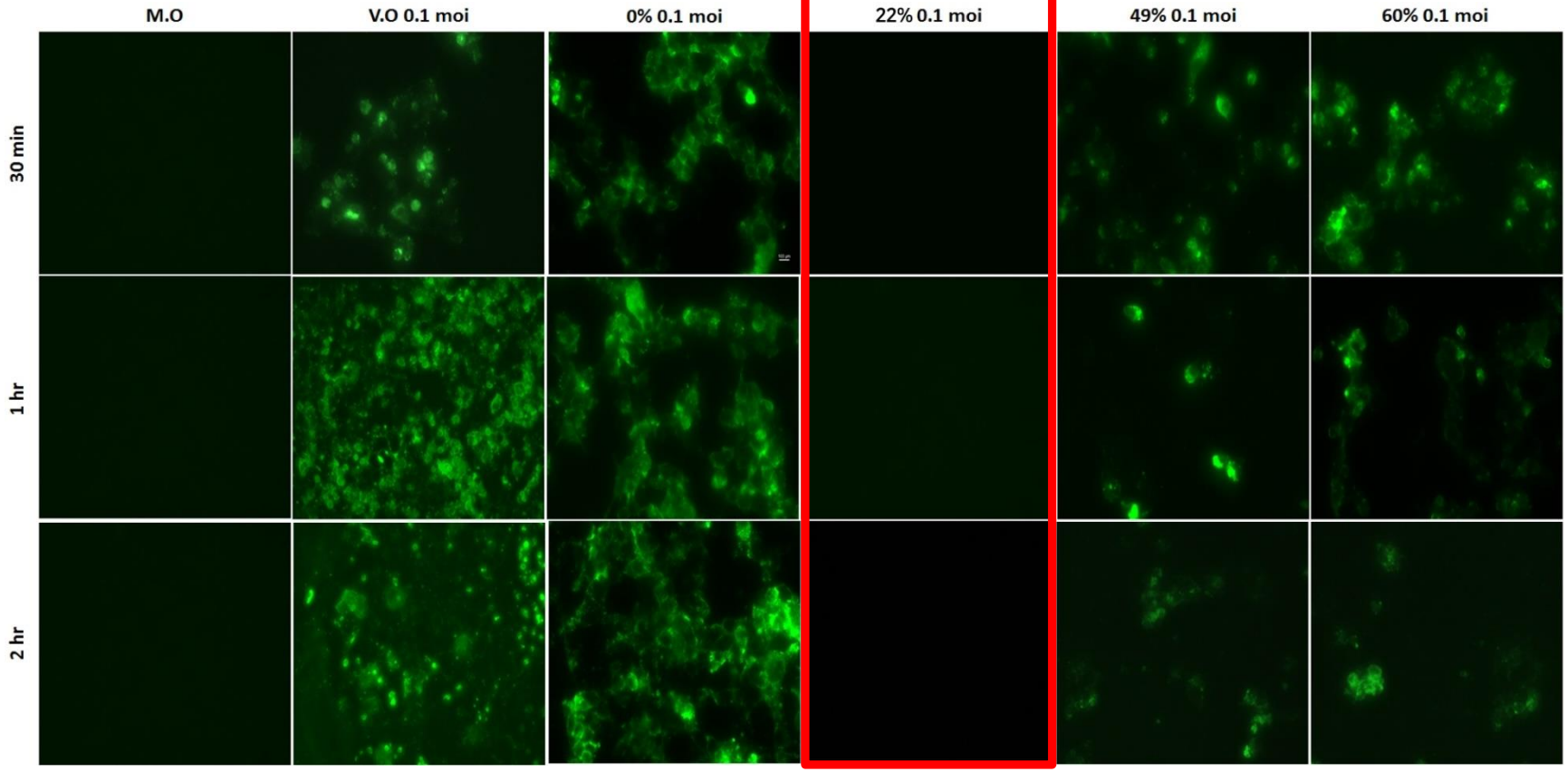
Mask material with 0% of copper was inactive against SARS-CoV2 at each different infection time points.

After 30 min of infection, **the 3D textured with copper-base mask materials with 22% of copper had nearly 100% and the 49% and 60% of copper coated general mask materials had approximately 80% of inhibitory effect against SARS-CoV2.**

RESULTS

3D textured with Copperline mask

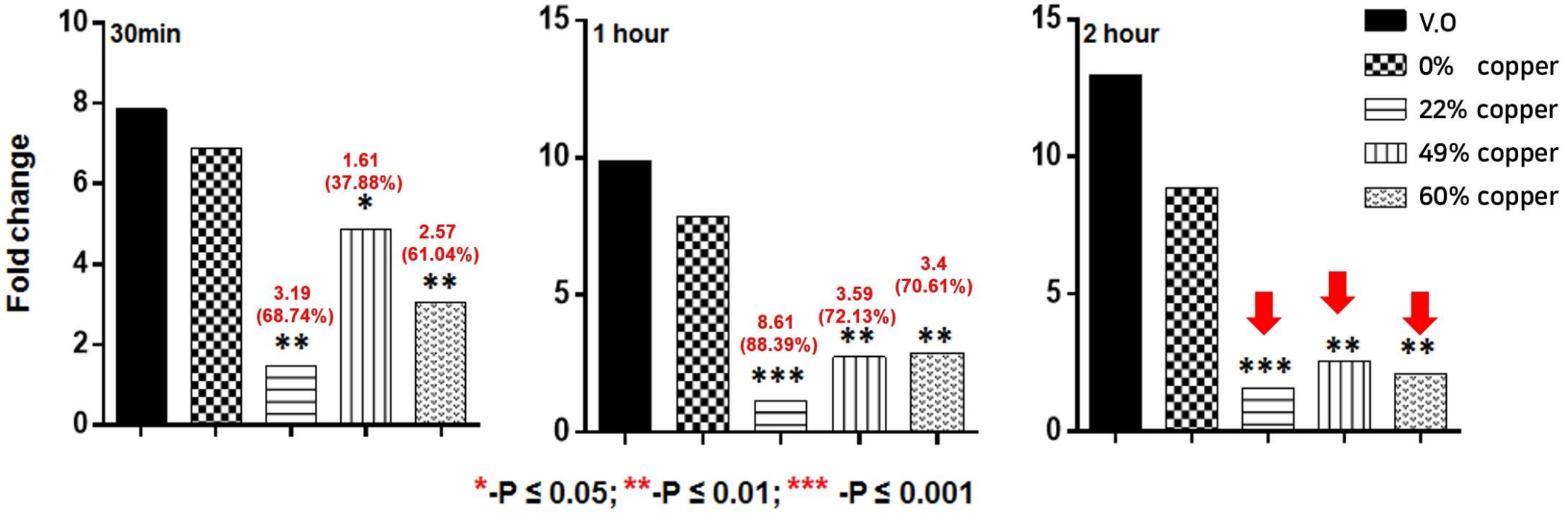
- **Immunofluorescence assay**



Compared to an intense green fluorescence signal that was observed 0% copper treatment group, there was almost no fluorescence signal at cells treated with the 3D textured with copper-base mask materials with 22% of copper to that of the control group.

RESULTS

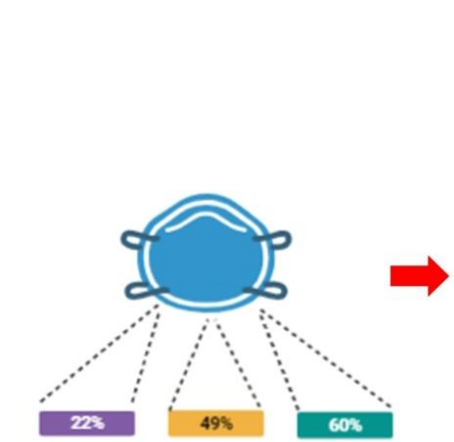
- **Quantitative Real-Time PCR**



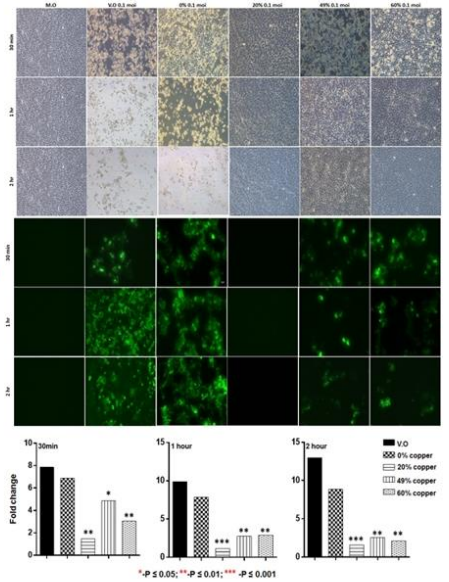
A pronounced decline in the relative expression of the SARS-CoV2 N gene was found when the virus was incubated with 22%, 49% and 60% copper membranes for 2 hours by 8.25 (87.88%), 5.03 (80.13%) and 6.18 (83.84%)- fold respectively.

CONCLUSION

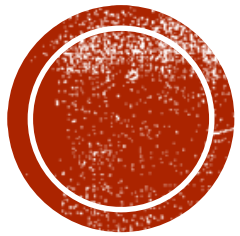
- The described data proves that use of copper in different percentages, actively inactivate SARS-CoV2 viruses and it seems to be effective and a low-cost strategy.
- Compared to control group, incorporation of copper increases cell viability by reducing the viral load and showed cell activity greater than 80%.
- **The 3D textured with copper-base mask materials with 22% of copper** had a higher capacity to readily kill the virions that remain in the mask. Due to its increased surface area, improves their microbicidal action via the interaction of copper ions with the virions that are entrapped in the mask or that come into contact with the surface of the copper impregnated outer surfaces of the masks.



Mask material with deferent copper percentages



In vitro studies showed increased cell viability by reducing the viral load



THANK YOU