The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has spread worldwide and placed countries in emerging, rapidly transforming situations. More than 88 million cases of COVID-19 and 1,926,625 deaths are reported to WHO as of 11 January 2021 (WHO, 2021). The SARS-CoV-2 has been detected in specimens from the respiratory tract, nasopharyngeal sites, and feces in COVID-19 patients (Wang et al., 2020). The viral transmissions can occur via close human-to-human contact or via contacting a contaminated surface. To reduce the risks of environmental contamination, a myriad of disinfectants/sanitizing agents/biocidal agents are available, but their effectiveness is likely to depend on many factors such as the concentration of the agent, the reaction time, temperature, and the organic load (Lin et al., 2020).

Although the effectiveness of representative sanitizers such as ethanol and sodium hypochlorite in deactivating SARS-CoV-2 has been studied (Aboubakr et al., 2020, Lin et al., 2020, Takeda et al., 2020), this information is limited to a few liquid disinfectants and does not obtain a comprehensive picture about the effects of solid materials on SARS-CoV-2.

Photocatalysts are sustainable, environmental friendly and potent disinfectants that generate free radicals (i.e. superoxide and hydroxyl radicals) when excited by light strikes. Thus, they are efficient biocides against many pathogens including bacteria, viruses and fungi (Habibi-Yangjeh et al., 2020, Yemmireddy et al., 2017), but, to our knowledge, their effects on SARS-CoV-2 have not been investigated. In this study, we examined the virucidal activity of a tungsten trioxide (WO₃)-based visible light-responsive photocatalyst, RENECAT™ against SARS-CoV-2 under visible light irradiation. To
exhibit antimicrobial effects, the visible light-responsive photocatalyst does not require excessive UV light irradiation that are harmful to humans, which allows us to be present even when the photocatalyst activates. By contrast, UV light-responsive photocatalysts require human evacuation from the target environments for its activation. Given the advantage of the former, it could be considered that our findings will contribute to the development of techniques for deactivating SARS-CoV-2 and decelerating its spread.

A human clinical SARS-CoV-2 strain JPN/TY/WK-521 was used in this study. All experiments were carried out in biosafety level (BSL) 3 facility at National Institute of Health Sciences. The virus was propagated in Vero E6/TMPRSS2 cell line (JCRB 1819) (Nao et al., 2019) cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Wako Pure Chemicals, Tokyo, Japan) and supplemented with 5% heat inactivated FBS (SAFC Biosciences, Lenexa, KS, USA) at 37°C in a humidified CO2 incubator.

To our knowledge, the virucidal activity of photocatalysts against SARS-CoV-2 has not been tested. In this study, we used the visible light responsive photocatalyst, RENECAT™ (Toshiba Materials, Kanagawa, Japan), which was mainly composed of tungsten trioxide (WO3). Four g/m² of the photocatalyst mixed with silica binder was coated onto 30 x 30mm soda-lime glass slides (AGC Inc., Tokyo, Japan). For the control, the glass slides were coated with silica binder alone, an auxiliary agent for the binding of the photocatalyst onto glass slides. To evaluate the photocatalyst’s virucidal activity, we followed the ISO 18071 protocol (https://www.iso.org/standard/64033.html) and illuminance guideline provided by Photocatalysis Industry Association of Japan (https://www.piag.gr.jp).

Briefly, 30 µL of the virus culture medium (pH 6.8) containing SARS-CoV-2 with 5% (v/v) FBS and a TCID50/mL of 5.93 to 6.24 log10 was placed on the photocatalyst-coated or a silica binder-coated (control) glass slide. Then, the slide was overlaid by a 25x25-mm VF-10 polypropylene plastic film sheet (Kokuyo Co., Ltd., Osaka, Japan) to allow a close contact between the virus and the photocatalyst. The slide was then placed in a 90-mm glass container with a wet filter paper to prevent evaporation. To remove UV light with wavelength <380 nm, an acrylic resin sheet (CLAREX N-169, Nitto Jushi Kogyo Co., Ltd., Tokyo, Japan) was placed between the lamps and the samples.

Viral samples on the glass slides that were coated with (i) the photocatalyst (n=4, photocatalyst group), (ii) no photocatalyst (n=4, glass group), (iii) only silica binder (n=4, binder group) were exposed to the fluorescence lamps (FL20SSW/18, Toshiba Lighting & Technology Corporation, Kanagawa, Japan), and simultaneously, viral samples treated with the photocatalyst were kept under the dark condition (n=4, photocatalyst w/o light group). After irradiation, the virus was recovered from glass slides by rinsing with 270 µL of serum-free DMEM, the viral suspensions and a tenfold serial dilutions were then incubated into Vero E6/TMPRSS2 cells for 3 d to calculate the TCID50 as described by Kärber (Kärber, 1931). FIG. 1A shows the experimental setup we used for this study.

One-way ANOVA test was performed with JMP 15.1 (SAS Institute, Cary, NC, USA) to analyze whether there is a significant difference in the virucidal activity between the groups. P values < 0.05 were considered statistically significant.

Firstly, we examined the stability of SARS-CoV-2 on the glass slides under a variety of experimental conditions including temperatures and incubation periods. The containers were placed under fluorescent lamps with an illuminance of 3,000 lux that were measured using digital illuminance meter IM-5 (TOPCON, Tokyo, Japan) for 6h at 20°C or 30°C (4 containers for each temperature) to select the temperature that has a lower effect on the viral titer.

Given that temperatures >30°C reduces the stability of coronaviruses on abiotic surfaces (Harmooshi et al., 2020), the viral stability was evaluated at 20°C and 30°C for 6 h, on the glass slide without the photocatalyst under visible light irradiation. After the 6h-incubation, the viral titer of 6.24±0.21 logTCID50/mL was reduced to 4.18±0.13 logTCID50/mL at 30°C, and to 5.55±0.31 logTCID50/mL at 20°C (FIG. 1B). The viral titer at 20°C was not significantly different from that of the control (0h) (P= 0.09) (FIG. 1B). Because at 30°C, the viral stability (4.18±0.13 logTCID50/mL) was significantly lower than the control (0h) (P< 0.01), we set up our experiments at 20°C to eliminate the effect of temperature on the viral titer while evaluating the photocatalyst’s virucidal effects.

In order to optimize the incubation periods, the viral suspensions were incubated on glass slides without the photocatalyst for 0h, 4h, 6h and 18h at 20°C under the visible light irradiation, followed by the viral titration. As shown in FIG. 1C, 18h of incubation resulted in the reduction of the viral titer to 4.18±0.22 logTCID50/mL, which were significantly different from that of the control (0h) (P< 0.01). The viral titer after the 4h- or 6h-incubation were relatively retained, represented as 5.81±0.18 logTCID50/mL or 5.35±0.22 logTCID50/mL, although they were significantly different from the control (0h) (P< 0.01) but no significant differences were shown between the 4h- and 6h-incubation periods (P= 0.17) (FIG. 1C). Our findings agree with a previous
FIG. 1. Optimization of the experimental conditions for the evaluation of virucidal activity of the photocatalyst against SARS-CoV-2. (A) Schematic representation of the experimental setup to evaluate the virucidal effects of visible light responsive WO₃ photocatalyst. Cool plate was used to adjust the temperature on the surface of the photocatalyst. (B) Evaluation of SARS-CoV-2 stability under different temperatures (20°C, 30°C). 5% FBS-containing SARS-CoV-2 suspension was incubated on the slide glass either at 20°C or 30°C for 6h under visible light irradiation (3,000 lux). (C) Evaluation of SARS-CoV-2 stability under different incubation periods (0h, 4h, 6h, 18h). 5% FBS-containing SARS-CoV-2 suspension was incubated on the glass slide for the different periods at 20°C. Error bars indicate mean ± S. D. (n=4 per a group). In sections B and C, one way ANOVA was used to analyze statistical difference in the viral titer between each of the temperatures (20°C and 30°C)/incubation periods (4h, 6h and 18h), and the control (0h). ** = P < 0.01.
study showing the longer time of incubation (i.e. 24h at room temperature) reduced the viral stability (Harmooshi et al., 2020). Based on these data, we setup our experiments at 20°C, with the incubation time of 6h to evaluate the virucidal activity of the photocatalyst.

The experiments showed that the photocatalyst under visible light significantly reduced the titers of SARS-CoV-2, from 5.93±0.38 logTCID<sub>50</sub>/mL to 3.05±0.25 logTCID<sub>50</sub>/mL (P<0.01). The viral titer was reduced to 5.55±0.25 logTCID<sub>50</sub>/mL in the absence of visible light (P< 0.46) (FIG. 2A). The viral stability on the silica binder alone (with no photocatalyst; binder), and on the glass slides in the absence of photocatalyst/silica binder under florescence light (glass) were 5.18±0.13 logTCID<sub>50</sub>/mL and 4.88±0.13 logTCID<sub>50</sub>/mL, and were significantly different from that on the photocatalyst (P< 0.01 or P= 0.04), respectively (FIG. 2A). These findings indicate that the silica binder under visible light or the presence of photocatalyst substances without light did not drastically affect the viral stability while photocatalyst under visible light significantly reduced the titer of SARS-CoV-2 during the experimental periods (6h). A previous study reported that UV at 254 nm could reduce the activity of SARS-CoV-1 (Damell et al., 2004). In this agreement, our comparative data of the photocatalyst treatment with or without visible light suggested that a part of the reduced viability of SARS-CoV-2 throughout the exposure on photocatalyst under visible light irradiation might be due to the light irradiation. It should be noted that 3,000 lux is generally achieved in indoor environments with direct sunlight exposure, as was suggested by the above-mentioned guideline from the Phosotcatalysis Industry Asssociation of Japan.

For immunoblot analysis, the viral spike protein within the recovered viral suspensions from the photocatalyst-coated glass slides with or without the visible light for 6h at 20°C, or from the glass slide without the exposure (0h), were analyzed. To detect the viral spike protein, rabbit anti-SARS-CoV-2 spike protein antibody (#ab273074, Abcam, Cambridge, UK) and goat HRP-conjugated anti-rabbit IgG (#4090-05, Southern biotech, Birmingham, AL, USA) were used as the primary or secondary antibody, respectively. HRP-conjugated anti-ß-actin antibody (#HRP-60008, Proteintech, Rosemont, IL, USA) was used to detect residual ß-actin from Vero E6/TMPRSS2 cell during preparation of viral suspension.

A decreased level of the viral spike protein and residual ß-actin protein (originated from Vero E6/TMPRSS2 cells) were observed after the 6h-treatment with the photocatalyst under visible light, compared with the sample before treatment (FIG. 2B). These data suggested the damage of viral surface protein and free proteins such as ß-actin by the photocatalyst treatment.

Furthermore, the changes of virion particle within the recovered viral suspensions from the photocatalyst-coated glass slides with or without the visible light for 6h at 20°C, were analyzed by transmission electron microscopy JEM-2010 (JEOL, Tokyo, Japan). Suspension aliquots (25 µL) were applied to 600-mesh carbon-coated copper grids, which were subjected to glow discharge. After absorption for 15 min, the grids were washed three times with water and treated for 30 s with 2% phosphotungsten acid/1% trehalose. The electron microscopic analyses indicated the reduced amounts of spike structural molecules on the viral surface after the photocatalyst treatment (FIG. 2C). A previous report demonstrated that one of the visible light responsive photocatalysts produced free radicals such as reactive oxygen species (ROS), thereby damaging viral surface proteins of MS2 bacteriophage (Li et al., 2016). SARS-CoV-2 spike protein localizes on the viral surface and binds to human host cell receptor, angiotensin-convert-ing enzyme 2 (ACE2) to establish an early course of infection (Scheller et al., 2020). As recently perspective by Sun and Ostrikov, 2020, future study on the kinetics of affinity of the viral spike protein to human ACE2 after the photocatalyst treatment would elucidate the impact of the photocatalyst treatment on the viral infectivity. Nevertheless, our results proved evidence for the virucidal effect of the visible light responsive photocatalyst against the SARS-CoV-2, with structural damage of viral surface protein.

Disinfection technologies at indoor environments are one of the key elements in avoiding the spreads of COVID-19, particularly for medical doctors and front-line healthcare workers in hospital (Ahn et al., 2020). Most liquid biocides such as ethanol, sodium hypochlorite and electrolyzed water demonstrate prompt anti-SARS-CoV-2 activity (Leslie et al., 2020, Takeda et al., 2020); however, those virucidal effects are short-term and become inactivated once contaminated with certain organic substances. By contrast, the photocatalytic techniques are expected to show relatively moderate but long-term virucidal activity because they are renewable (Tahir et al., 2020). In this study, we demonstrated the virucidal activity of the photocatalyst under a controlled experimental condition (6h-light exposure at 20°C). Several factors can affect the efficiency of photocatalyst to inactivate pathogens (i.e. pH, temperature, catalyst loading, light intensity and wavelength) (Oxford et al., 2014), but our experiments were conducted under conditions to mitigate the effects of other factors, and only evaluated the virucidal activity of the WO<sub>3</sub> photocatalyst against the SARS-CoV-2. In summary, our data suggest that the visible light-responsive photocatalyst can (possibly) be used to disinfect and prevent the viral
FIG. 2. The visible light responsive photocatalyst exhibits virucidal activity against SARS-CoV-2. (A) Results of SARS-CoV-2 inactivating activity of the photocatalyst. 5% FBS-containing SARS-CoV-2 suspension on the photocatalyst was exposed to the visible light for 6h at 20°C (photocatalyst + Light) or kept under dark conditions (photocatalyst w/o Light). Also, the viral titer was measured on the silica binder (binder) or glass slide (slide alone). Error bars indicate mean ± S. D. (n=4 per group). ANOVA test was used to analyze statistical significance between the groups. * = P < 0.05, ** = P < 0.01. (B) Western blot analysis for the detection of the viral spike protein as well as β-actin protein, in the viral suspensions throughout the photocatalyst treatment. Lane M, molecular weight marker; 1, viral suspension before exposure (control); 2, viral suspension after 6h-incubation on the photocatalyst without light exposure (photocatalyst w/o light); 3, viral suspension after 6h-incubation on the photocatalyst under visible light irradiation (photocatalyst). (C) Representative electron micrographs of SARS-CoV-2 with or without treatment of the photocatalyst for 6h. Left panels show the images of virus treated without the photocatalyst. Right panels represent the virus treated with the photocatalyst. Bars indicate 100nm scale.
spread in indoor environments in the presence of visible light. Future studies would be required to evaluate the effectiveness of the photocatalyst in the real world and clarify how long the photocatalyst could retain virucidal activity against SARS-CoV-2 throughout a continuous viral exposure. Moreover, the photocatalyst's antiviral effects on different surfaces, such as plastic, stainless and clothes should be further investigated, because different materials affected the stability of influenza A virus differently (Oxford et al., 2014) and they may have similar effects on SARS-CoV-2. Lastly, exploring viral molecules and mechanisms that are involved in the stability of SARS-CoV-2 will detect potential target molecules and pathways for deactivating this virus.

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ABBREVIATIONS

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane serine protease 2; COVID-19, coronavirus disease 2019; TCID50, 50% tissue culture infective dose; FBS, fetal bovine serum; WHO, World Health Organization

REFERENCES


