Inactivation of Goose Parvovirus, Avian Influenza Virus and Phage by Photocatalyst on Polyethylene Terephthalate Film under Light Emitting Diode (LED)

Tomomi HASEGAWA1), Miho TAMURA1), Keisuke SATOH1), Misato TSUJIMURA1), Akinobu KAWAMURA1), Chanathip THAMMAKARN1), Hakimullah HAKIM1), Sakchai RUENPHET2) and Kazuaki TAKEHARA1)*

1)Laboratory of Animal Health, Department of Veterinary Medicine, Tokyo University of Agriculture and Technology, 3–5–8 Saiwai-cho, Fuchu, Tokyo 183–8509, Japan
2)Virology and Immunology Department, Faculty of Veterinary Medicine, Mahanakorn University of Technology, 140 Cheum-Sampan Rd. Nong Chock, Bangkok 10530, Thailand

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ABSTRACT. The inactivation effect of a novel photocatalyst on polyethylene terephthalate film on goose parvovirus (GPV), avian influenza virus (AIV) and Qβ phage was evaluated. Under a light emitting diode (LED) light (range 410–750 nm), GPV was inactivated by irradiation at 1,000 lux for 6 hr, while AIV and Qβ phage were inactivated by irradiation at 150 lux for 2 hr. These data suggest that this new photocatalyst can potentially be used as one of the materials to inactivate viruses in the indoor environment and help us to prevent viral infectious diseases through indirect contact.

KEYWORDS: goose parvovirus, LED, photocatalyst, visible light


Control of infectious diseases has become increasingly important, because of the many outbreaks of zoonoses, such as pandemic influenza, severe acute respiratory syndrome (SARS) and Nipah virus infection, around the world. Not only these infectious viruses but also agents that appear sporadically like Norovirus, Campylobacter, Salmonella and hand, foot and mouth disease (HFMD) virus should be controlled. It has become necessary at facilities that have high-risk populations, for example, hospitals, kindergartens and nursing homes, to ensure control of pathogens.

Photocatalysts is the generic name for materials that are excited with light strikes and generate free radicals like superoxide and hydroxyl radicals that decompose organic matter of microorganisms [11]. They have attracted attention as environmentally friendly substances for inactivating pathogens. The most well-known photocatalyst is titanium oxide (TiO₂), and many reports have shown its disinfection effect towards various microorganisms under ultraviolet (UV) radiation [1, 3, 4, 5, 8, 13, 22]. Recently, several papers on TiO₂ under visible light radiation were published, but the methods they described required a long incubation period (24 hr) to inactivate viruses [16]. A novel photocatalyst, iLUMiO, developed by Sumitomo Chemical Co., Ltd. (Tokyo, Japan) is a platinum-supported tungsten oxide (Pt-WO₃). It has already been reported to have inactivation activity towards avian influenza virus (AIV) under visible light conditions without UV radiation [21].

Among pathogens used to verify the effectiveness of disinfectants and materials to inactivate viruses, parvovirus is increasingly being used. Parvovirus is one of the most resistant viruses among non-enveloped viruses known to be highly resistant and has been investigated using physical and chemical treatments [2, 6]. So, it is considered to significantly increase the reliability of tests for such an effect when validating the efficacy of materials that can inactivate viruses. We used a goose parvovirus that demonstrated high resistance in one of our previous experiments [19].

In addition, AIV strongly interests many people [17, 18], and Qβ phage is being used in many studies. Qβ phage, one of the enterobacteriophages, infects Escherichia coli through E. coli’s pili [7]. This phage belongs to Leviviridae, Allolevivirus, a non-enveloped positive strand ssRNA virus, with a diameter of 20–30 nm and an icosahedral structure. Furthermore, it is labile against UV light and chemicals [7, 12]. Also, due to the fact that it is easily handled as a biosafety level (BSL) 1 virus and can be used in a BSL1 facility, it could be a model for test materials to inactivate viruses. So far, CuxO/TiO₂ has been investigated to determine, if it can inactivate Qβ phage [15].

In the present study, the effect of a new photocatalyst prepared on film together with a light emitting diode (LED) in the absence of UV radiation was verified using GPV, AIV and Qβ phage. To date, there has not been enough reports investigating the photocatalytic effects using visible light conditions considered to be suitable to indoor life. When using a photocatalyst indoors, UV radiation is not appropriate, so visible light as well as LED light, the major forms of indoor lighting, should be used in the experiments. Here, we show the potential of Pt-WO₃ for indoor usage by using visible and LED lights for light striking.

A photocatalyst based on platinum-loaded tungsten oxide (Pt-WO₃), named iLUMiO coated on polyethylene terephthalate (PET) film at a concentration of 1 g per 1 m² and then cut into 5 × 5-cm pieces was kindly supplied by ©2013 The Japanese Society of Veterinary Science
Goose parvovirus (GPV) strain-IHI [20] and avian influenza virus (AIV) a/northern pintail/Miyagi/1472/08 (H1N1) [10] were from our stock, while Qβ phage (NBRC20012) and E. coli (NBRC106373) were purchased from the Biological Resource Center (NBRC), National Institute of Technology and Evaluation (Chiba, Japan). GPV was grown and titrated in Muscovy duck embryo fibroblasts (MDEFs) prepared from 14-day-old Muscovy duck embryos. The growth medium (GM) for MDEFs was prepared with Eagle’s minimum essential medium (MEM, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with penicillin 100 units/mL, streptomycin 100 µg/mL, amphotericin B 0.5 µg/mL, 4 mM L-glutamine and heat-inactivated 5% (v/v) fetal bovine serum (FBS). Maintenance medium (MM) was prepared like the GM without FBS. MDEFs were prepared in GM and incubated in a humidified environment at 37°C in a CO₂ (5%) incubator. After the MDEFs occupied a full sheet, the GM was replaced with MM and GPV was inoculated. Madin-Darby canine kidney (MDCK) cells were used for AIV. MDCK cells were passaged and maintained in GM and MM, respectively. Qβ phage was maintained in phosphate buffered saline (PBS), and E. coli was cultivated in Luria-Bertani (LB) medium (1% Bacto Tryptone, 0.5% Bacto Yeast Extract and 1%NaCl, pH 7.4) and titrated on LB agar (LB containing 1.5% Bacto Agar) as described [14, 23].

The activity for virus inactivation of the photocatalyst was evaluated according to a film adhesion method using an assay based on that described for inactivation activity of bacteria described by the International Organization for Standardization [9]. In short, 100 µl of viruses, adjusted to 10⁵–⁷ TCID₅₀/ml or 10⁵ plaque-forming units with PBS, was spotted on the photocatalyst (for PET film, the film was placed on a 5 × 5-cm glass plate with the photocatalyst side up), and then 4 × 4-cm VF-10 polypropylene plastic film sheet (60-µm thick, Kokuyo Co., Ltd., Osaka, Japan) was laid on the sample to allow close contact of the virus and the photocatalyst. The plate was placed in a 90-mm plastic dish with filter paper sheets containing 3 mL of water, and the dish was covered with a 10 × 10-cm glass plate to retain moisture. Then, the dish was placed under fluorescent lamps (FL20ss W/18, NEC Lighting, Ltd., Tokyo, Japan) or an LED light (SUMILOOK, Sumimoto Chemical Co., Ltd.), with a luminous intensity of 1,000 or 150 lux for 2 and 6 hr. In the case of fluorescent lamps, an acrylic resin sheet N-113 (Nitto Jushi Kogyo Co., Ltd., Tokyo, Japan) was also placed between the lamps and samples to remove UV light with wavelengths <410 nm. The photocatalyst-coated glass plate was also evaluated for GPV under dark conditions in a stainless steel box. At the same time, PET films that were not coated with a photocatalyst were prepared as negative controls. After irradiation, the virus was recovered from PET film by rinsing with 1 ml of MM (for AIV and GPV) or 900 ml of PBS (for Qβ phage) and collected into an aseptic plastic bag. Each recovered virus was titrated on cells. To confirm the recovery of viruses from the films, viruses spotted on the films were recovered immediately after the cover films were placed, without incubation. For each virus, anti-viral activity was evaluated in triplicate. All experiments were carried out at room temperature (25°C).

GPV and AIV were inoculated to MDEF or MDCK cells sheeted on a 96-well microplate with 100 µl of MM. For GPV, the plates were incubated at 37°C and observed for virus-induced cytopathic effect (CPE) for 7 days, and on the last day, the endpoint cell viability was assayed by crystal violet staining [20]. In the case of AIV, the plates were incubated as done for GPV and observed for virus-induced CPE, and the hemagglutinin (HA) activity of the culture supernatant was checked with 0.5% chicken red blood cells (CRBCs) at 3 dpi. Titer was calculated by Behrens and Kaerber’s method as the TCID₅₀/ml [10]. The iLUMiO photocatalyst was also evaluated using Qβ phage by applying a method similar to that described previously [14, 23]. Nine hundred microliters of Qβ phage and 100 µl of E. coli and LB containing 1.0% Bacto Agar were poured onto plates containing 1.5% Bacto Agar. The plates were incubated at 37°C overnight. Plaques were counted the following day, and titer was determined.

Viral titers in the representative data are shown in Table 1. AIV was reduced to an undetectable level within 2 hr by the photocatalyst on the PET film with 150 and 1,000 lux of LED light. GPV was reduced partially with 1,000 lux LED light exposure for 2 hr and less than detectable for 6 hr. For GPV, the photocatalyst prepared on a glass plate was also evaluated, but the results were similar to those obtained on PET film (data not shown). Under dark conditions, GPV treated on the glass plate with the photocatalyst for 6 hr was partially inactivated (10-fold reduction; data not shown). For AIV and GPV, fluorescence light without UV was used to inactivate viruses, but the results were similar to those obtained with LED light (data not shown). The titer of Qβ phage reached around 10¹¹ PFU/ml. Qβ phage was diluted 100 times with PBS and then applied the film. The titer decreased by more
than 10,000-fold from $10^8$ to $10^5$ PFU/ml within 2 hr under 150 lux of LED light (Table 1).

A photocatalyst based on TiO$_2$ requires UV light for a long period of time to kill viruses or bacteria [1, 3, 5]. Sang et al. [16] reported that TiO$_2$ could inactivate non-enveloped viruses, including rotavirus, astrovirus and calicivirus with visible light irradiation after exposure for 24 hr. We have already demonstrated that the novel photocatalyst based on platinum-loaded tungsten oxide (Pt-WO$_3$), named iUMiO, coated on glass plates showed antiviral activity against HIV H1N1 under visible light within 2 hr [21].

In the present study, we confirmed that the photocatalyst could be used on a film under LED light and that it showed antiviral activity against GPV, HIV and QB phage. Paroviruses are quite resistant against physicochemical treatments [2, 6, 19]. These days, there are many reports on evaluation of disinfectants using paroviruses [2, 6]. GPV, a pathogen that belongs to the Paroviridae family, also showed resistance against many disinfectants and physical treatments [19]. The fact that this photocatalyst could inactivate QB phage and goose parovirus suggests a further possibility to inactivate other non-enveloped viruses.

To date, use of photocatalysts has been limited, because they need to be activated by UV rays and have mostly been used outside like on walls or windows exposed to sunshine. But, the present novel photocatalyst has the potential to display its antiviral activity as a wallpaper or coating for indoor furniture in combination with radiation from visible or LED light. Application of this photocatalyst is considered effective for infectious diseases when processing equipment used for high-risk groups like children or the elderly and for hospital infections, which are regarded as an important problem. In the hospital, there is a report indicating that a pathogen dried with a hotbed, such as blood, becomes resistant, which promotes infection [6]. For cases like this, applying the photocatalyst to equipment in the hospital would be effective.

REFERENCES


