Inactivation of 2009 Pandemic Human Influenza A Virus H1N1 by Photocatalyst Under UV Irradiation

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A large-scale pandemic by human influenza virus H1N1 in 2009 caused severe health, social, and economic impacts. In this study, a photocatalyst technology based on TiO2, was evaluated for inactivation of a human influenza virus H1N1 isolated from a patient. The virus titer was reduced by 103.16-fold within 24 h and more than 104.31-fold inactivation within 48 h and 72 h. These results suggest that the tested photocatalyst technology based on TiO2 can be used for reduction of influenza A virus adherence to other surfaces with Hizen-s inside diverse buildings, enabling effective control of its indirect contact infection. The photocatalyst is expected also to reduce level of the aerosol transmission of the virus.

Key Words: H1N1, Inactivation, Photocatalyst

Influenza, an infectious disease affecting birds and mammals, is caused by RNA viruses of the family Orthomyxoviridae (influenza viruses, IFV). In humans, common symptoms of this disease include chills, fever, pharyngitis, muscle pain, severe headache, coughing and weakness. Influenza causes pneumonia in more serious cases, which can be fatal, particularly in young children and the elderly. Influenza has a history of fatality during seasonal epidemics (1). In the past, influenza had been spread around the world during seasonal epidemics, resulting in the deaths of hundreds of thousands annually - millions during pandemic years (2). It spreads rapidly around the world in seasonal epidemics, affecting 10~20% of the total population (3).

In March 2009, case of influenza-like illness in Mexico caused by a novel H1N1 virus containing genes from swine, avian, and human influenza strains were reported. Within several weeks, 2009 H1N1 disseminated rapidly and was the predominant influenza strain globally (4). On 11 June 2009, WHO raised the level of influenza pandemic alert from phase 5 to phase 6 (5). This influenza virus H1N1 has since spread over more than 208 countries, claiming the life of more than 11,516 people.

From May 2009 to January 2010, a total of 740,835 patients in South Korea were reported as having pandemic (H1N1) 2009 virus infection. A total of 225 patients (0.03%) died of disease related to pandemic (H1N1) 2009 (6).

Hydroxyl radicals generated from photocatalyst reactions were considered to play a significant role in microbial inactivation (7). Since photocatalytic properties of titanium dioxide (TiO2) were first reported by Fujishima and Honda (1972), most of the publications dealing with anti-microbial photo-inactivating agents have evaluated photocatalysts based on TiO2 (7~9). However, a study on inactivation of
human viruses has not yet been performed in South Korea. In this study, a photocatalyst based on TiO₂, designated as Hizen-s developed by Kukje Telecommunication Co., Ltd. (Gyeonggi-do, South Korea), was evaluated for inactivation of a human influenza virus H1N1 isolated from a patient.

Mardin-Darby canine kidney (MDCK) cells (ATCC, Manassas, VA, USA) were grown in minimum essential medium (MEM; Gibco-Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco-Invitrogen). The human influenza virus H1N1 was propagated by infection of MDCK cells in the presence of 2 μg/ml tolylsulfonyl phenylalanyl chloromethyl ketone (TPCK)-trypsin (Sigma-Aldrich) at 37°C. Viral stocks were stored at -70°C and titrated by plaque assay. Confluent cultures of MDCK cells in 6-well plates were infected with human influenza virus H1N1 at 37°C for 2 h. MDCK Cells were washed with PBS and overlaid with serum-free MEM containing 0.5% carboxymethyl cellulose and 2 μg/ml TPCK-trypsin in the presence or absence of increasing amounts of the test compounds. After 2 days, the plaque number was counted by staining with 0.01% Neutral red solution. Virus titers were determined to be $1 \times 10^7$ pfu/ml.

Two-hundred micro-liter of virus ($1 \times 10^6$ pfu/ml) was spread on 100 mm cell culture dish containing culture medium of 2 ml and exposed to the TiO₂ generated by Hizen-s for 24 h, 48 h, and 72 h. The viruses were collected from the cell culture dish depend on the exposure time. The collected viruses were quantified by plaque assay. Inactivation levels were determined by Log reduction. All of the tests were performed by duplicate. Negative controls are performed without UV irradiation.

Negative control virus titer was constantly around $2.1 \times 10^4$ pfu/ml, whereas the virus titer on the photocatalyst based on TiO₂ was rapidly reduced after 24 h post-infection. The virus titer was showed $1 \times 10^1$, 0, and 0 pfu/ml for 24 h, 48 h, and 72 h, respectively (data not shown). Even the virus titer was reduced by $10^{3.16}$-fold within 24 h and more than $10^{4.31}$-fold inactivation within 48 h and 72 h (Table 1). Normally, inactivation of viruses was considered effective if the virus titer was decreased by >3.0 log10 i.e. 99.9%.

These data presented in this study suggest that the tested photocatalyst technology based on TiO₂ can be used for reduction of influenza A virus adherence to other surfaces with Hizen-s inside diverse buildings, so that indirect contact infection of influenza could be effectively controlled. The photocatalyst could as well reduce the level of the aerosol transmission.

### REFERENCES


7) Cho M, Chung H, Choi W, Yoon J. Linear correlation

<table>
<thead>
<tr>
<th>Exposure time</th>
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<th>Standard deviation</th>
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