Inactivation of SARS virus by photo catalyst

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1. Preface

New type of pneumonia (SARS: Severe Acute Respiratory Syndrome) that infection is reported in dozens of countries of the world. This virus might be not only the droplet infection but also the air infection. An action to the prevention of in-hospital infection is demanded from reports that most of SARS patients were contagious in hospitals in many foreign countries.

On the other hand, as for the photo catalysis of TiO2 that many academic reports that TiO2 has strong antibacterial or anti-virus power for pathogens such as an influenza virus or the MRSA, but I cannot still find any detail report about the anti-virus characteristics of the titanium oxide for the SARS virus. Noritake has experiences to process that performed an action to the air cleaning with a photo catalyst filter made by ceramics and, at this occasion, we inspected the inactivation effect of the SARS virus by TiO2 by a collaboration with Tokyo Medical and Dental University. Furthermore, we examined mechanism of the virus inactivation by the photo catalyst with Sindo virus (SV). We manufactured a photo catalyst filter and it was equipped to a real air cleaner and we confirmed removal performance of floating bacteria in the room.

2. An experiment method

The virus cultures used SARS-CoV, Frankfurt culture (FFM-1), and virus multiplication, a plaque assay used cell of strain that is Vero of the African green monkey kidney origin. Virus solution of 200μl dropped to a photo catalyst board (the soda glass which coated Tio2 slurry made in Noritake Company) of 30mm φ and it was covered by a cover glass (ultraviolet rays' transmission rate 87%) and irradiated ultraviolet rays (a black light) of 3mW/cm2.

After irradiation, the photo catalyst glass board was washed by phosphoric acid salt buffering solution (PBS) including 1ml 0.1%BSA (cow serum albumin FV, Sigma) and the cleaning fluid was diluted appropriately.

The virus dilution fluid 200μl is inoculated to a Vero cell (the 90% single-layered formation) which removed culture fluid on six hole culture plates. Furthermore, it was stirred to protect dry prevention of the cell surface for every 10 minutes at room temperature and the virus is infected to it for 60 minutes.

After the virus infection, it was multistoried by D-MEM (which became non-motion at 56 □ with 5%FBS for 30 minutes) including the 0.8% methyl cellulose (4,000centipores, Sigma) and left at rest in a CO2 incubator.

After incubated for 4 to 5 days, methyl cellulose fluid culture was removed and washed by PBS and

dyed with 2.5% crystal violet liquid (2,5%, 30% ethanol, 1% ammonium oxalate). After dying, removed the dyeing liquid and washed by PBS twice, and sterilized by UV rays light and dried in a security cabinet. Then, it was taken out from the cabinet and the number of the plaque was counted and calculated quantity of virus of the whole cover quarantine inspection in pfu/ml. In addition, we observed the form change of the virus particle by an electron microscope after doing a same test for refined SV.

3. Results and Consideration

Figure1, it shows the inactivation rate of SARS virus in each irradiation time of ultraviolet rays in figure 1. (▲: used a photo catalyst board) Virus density fell down with irradiation start remarkably and reached 1x100pfu/ml 10 minutes later. This is inactivation rate of 99.9% in comparison with □ (did not use photo catalyst and only ultraviolet irradiation), and. it was less than detection limit (50pfu/ml) more than 15 minutes in irradiation time.

In addition, the inactivation rate was 99.99% in 15 minutes when I calculated the inactivation rate of SARS virus considering antivirus reaction of photo catalyst and ultraviolet rays. According to above test results, we could say that the photo catalyst board could inactivate SARS virus under ultraviolet rays of 3mWcm2 in a short time.

Figure 2 are results of the inactivation examination of SV. It was inactivated like SARS virus, and the inactivation rate became 99.999998% by irradiation of 15 minutes.

Figure 3 shows a TEM photograph of SV after each ultraviolet irradiation. We could confirm the spikes which were a part of envelope of the virus by ultraviolet rays' non-irradiation and could not recognize it in case of the virus that was irradiated ultraviolet rays. In addition, we thought that the dyeing liquid comes into the inside of the virus inside with the photograph of 10 minutes in ultraviolet irradiation time.

When virus infect a host cell, the spikes take an important role to adsorb a host cell specifically and we thought that the inactivation of the virus was achieved by a photo catalyst effect that destroyed the envelopes such as spikes and the infection of the virus is restrained. In addition, we consider that same mechanism occurred on SV because SARS virus has spikes and it was inactivated.

The next step, we made an air cleaner that has a ceramic filter covered TiO2 (a product made in Noritake) by a method of this photo catalyst board, and with the ultraviolet rays strength manufactured 3mW/cm2 like the examination same as above virus test. We diverted a room air to this air cleaner with the wind velocity of 5m/s and measured the number of the floating bacteria of the inhalation side and the discharge side-related (figure 4). As the result, we got that 94.5% decrease as for the number of the floating-related bacteria in the filter passage.

If this equipment works in a space (45m3 living room with six persons) for one hour, we confirmed that floating living bacteria shall be able to go down to about 1/6. Because of the above-mentioned result, it is suggested that virus / bacteria including SARS virus floating in the air can remove and inactivate effectively by the photo catalyst filter.

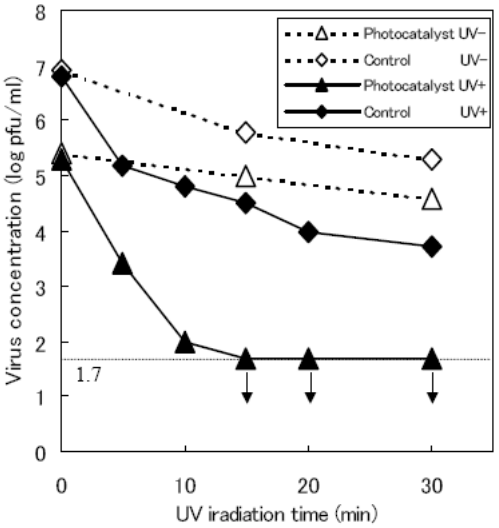


図 1. 各紫外線照射時間における SARS 濃度測定

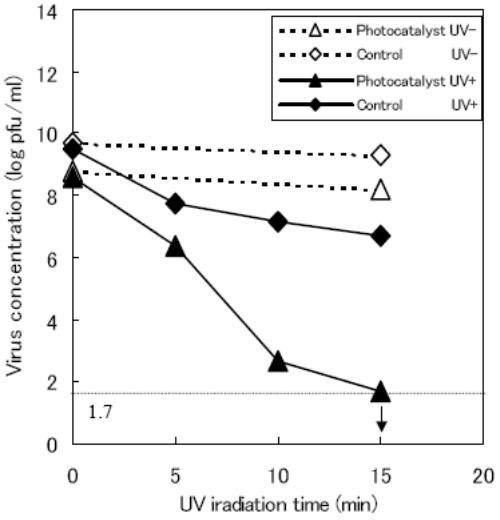
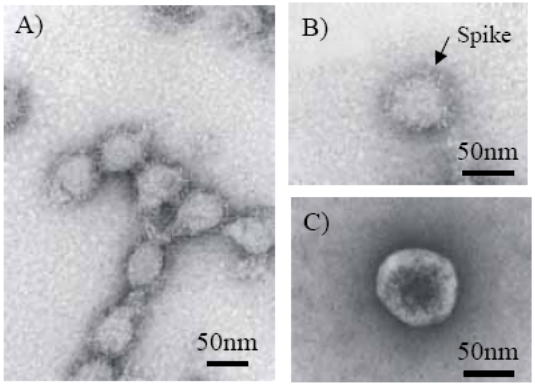


図 2. 各紫外線照射時間における SV 濃度測定



A) 実験に使用した SV
B) 紫外線未照射
C) 紫外線 10 分照射

図 3. TEM 観察結果

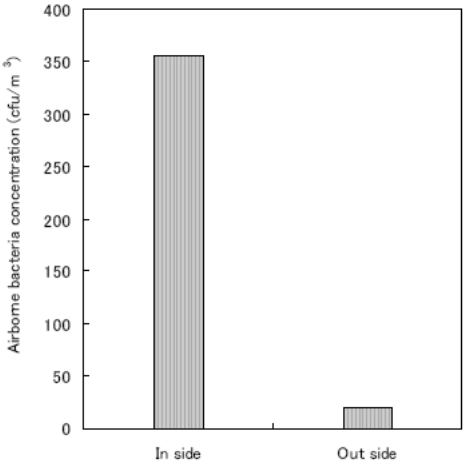


図 4. 空気清浄機 in-out 浮遊菌濃度

Figure 1. Measures SARS density in each ultraviolet irradiation time

Figure 2. Measures SV density in each ultraviolet irradiation time

A) SV which was used for an experiment

B) Ultraviolet rays non-irradiation

C) Ultraviolet rays 10 minutes irradiation

Figure 3. TEM observation result

Figure 4. Air cleaner in-out floating bacteria density

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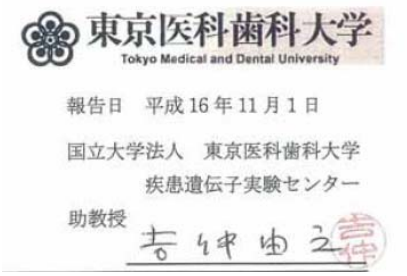

Examination Report

Tokyo Medical and Dental University

Date:　1. Nov. 2006

National university Tokyo Medical and Dental University disease gene experiment center

Assistant Professor Yoshinobu Yoshiyuki

[Examination item]

An SARS virus inactivation examination by the photo-catalyst effect [Examination virus culture]

SARS-CoV Frankfurt culture (FFM-1)

[Examination condition]

Test sample　　: A) 30mm Ø stick made by photo-catalyst (produced by Norotake Co. Ltd)

Light　　: A black light lamp (10W)

Irradiation condition: Irradiate the samples from distance of 1.5mm (ultraviolet rays' strength: 2.8mw/cm2)

Test method　　: Drip virus solution 20μl on a sample, cover it by cover glass to keep the surface in wet condition and irradiate ultraviolet light in scheduled time. After irradiation, the sample and cover glass were washed by 0.1% albumin PBS (a phosphoric acid buffering salt solution) and diluted it appropriately, and the virus dilution 200μl was inoculated for a Vero cell (the cell culture is 90% single-layered formation of the African green monkey's kidney origin) that removed cultivate solution on six holes culture plates. The next step, for dry prevention of the cell surface, the virus solution was stirred at room temperature every 10 minutes and is contagious for 60 minutes.

After infection, it was multistoried by D-MEM (which became non-motion condition by added 5% FBS at 56 degrees Celsius for 30 minutes) including the 0.8% methyl cellulose and was rested in a CO2 incubator.

Four or five days later, it was removed methyl cellulose component culture solution and dyed it with 2.5% crystal violet solution for five minutes after washing in PBS one time.

Then, removed the dying solution and washed it in 0.1% albumin component PBS two times, and dry and irradiate by UV light in a clean bench. After that, counted the number of the plaque and calculated quantity of virus.

[Test result]

*1: The photo-catalyst sample could inactivate SARS virus 99.98% by irradiating ultraviolet rays for 10 minutes to compare to blank sample (ultraviolet irradiation only, without photo-catalyst). (Table 1)

Table 1. SARS virus inactivation test result

A document	Quantity of virus（X1000pfu/ml）					
	0	5	10	15	20	30
Blank *2 under shade	1000	-	-	650	-	
Test Sample under shade	200	-	-	100	-	40
Blank ultraviolet irradiation	1000	150	70	30	10	5
Test Sample ultraviolet irradiation	200	2.5	0.1	<0.05	<0.05	<0.05

* Calculation method of inactivation rate = B / A x 100 of one inactivation rate (%)

* Two blanks: 30mm φ glass pieces