BIOMEDICAL AND ENVIRONMENTAL SCIENCES **18**, 176-180 (2005)

Efficient and Quick Inactivation of SARS Coronavirus and Other Microbes Exposed to the Surfaces of Some Metal Catalysts[1]

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Objective To study the two metal catalysts Ag/Al_2O_3 and Cu/Al_2O_3 that interdict the transmission pathway for SARS and other respiratory infectious diseases. **Methods** Two metal catalysts Ag/Al_2O_3 and Cu/Al_2O_3 were pressed into wafers. One hundred μL 10^6 $TCID_{50}$/mL SARS-CoV, 100 μL 10^6 PFU/mL recombinant baculovirus expressing hamster's prion protein (haPrP) protein and roughly 10^6 *E. coli* were slowly dropped onto the surfaces of the catalyst wafers and exposed for 5 and 20 min, respectively. After eluted from the surfaces of wafers, the infectivity of viruses and propagation of bacteria were measured. The expression of PrP protein was determined by Western blot. The morphological changes of bacteria were observed by electronic microscopy. **Results** After exposure to the catalysts surfaces for 5 and 20 min, the infectivity of SARS-CoV in Vero cells and baculovirus in Sf9 cells dropped down to a very low and undetectable level, and no colony was detected using bacteria culture method. The expression of haPrP protein reduced to 21.8% in the preparation of Sf9 cells infected with recombinant baculovirus exposed for 5 min and was undetectable exposed for 20 min. Bacterial membranes seemed to be cracked and the cytoplasm seemed to be effluent from cell bodies. **Conclusion** Exposures to the surfaces of Ag/Al_2O_3 and Cu/Al_2O_3 destroy the replication and propagation abilities of SARS-CoV, baculovirus and *E. coli*. Inactivation ability of metal catalysts needs to interact with air, utilizing oxygen molecules in air. Efficiently killing viruses and bacteria on the surfaces of the two metal catalysts has a promising potential for air-disinfection in hospitals, communities, and households.

Key words: Metal catalysts; Inactivation; SARS-CoV; Baculovirus; *E. coli*; Infectivity

INTRODUCTION

The outbreak of severe acute respiratory syndrome (SARS) involved more than 30 countries and areas in Spring 2003, affecting 8 437 individuals and resulting in 813 deaths[1]. It is believed that a novel coronavirus (CoV), with roughly 29 700 bp nucleotides in the context of whole genome[2], is the causal agent for SARS, based on the studies of morphology, epidemiology, serology, molecular biology, *etc*.[3-6]. The epidemiological data have strongly revealed that SARS transmissions are mainly family-cluster and hospital-cluster[7], in which close contact, including droplets, fomites and direct contact, is believed as the most possible modes. Other possible modes, e.g. airborne and fecal-oral chain, have been proposed as well, based on the observations in Amoy Garden in Hong Kong.

Recent studies showed that SARS-CoV was quite stable in human specimens and in environments. The infectivity of SARS-CoV in cell cultures showed little change in human blood, sputum and feces, as well as on the surfaces of several materials commonly used in households and hospitals after exposed for 2 or 3 days[8]. Moreover, prolonged virus detection in samples of blood, feces and respiratory excretions from SARS patients increased the frequency for SARS-CoV transmission[1, 6].

For inactivation of SARS-CoV in environment,

[1]This work was supported by the National High-Technology Research and Development Program of China (863 Program) 2003AA208402 and 2003AA208201.

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0895-3988/2005
CN 11-2816/Q

large amounts of chemical disinfectors were widely used in hospitals, households and even communities in the epidemic areas, resulting in great public concerns both on human health and on the environment. Although ultraviolet (UV) irradiation was confirmed to be efficient to eliminate the infectivity of SARS-CoV *in vitro*[8], poorly penetrating ability and harmfulness for humans limited its usage. More recently, surface science and catalysis studies showed that oxygen molecules could be adsorbed and dissociated into active oxygen atoms on some metal surfaces[9-10], and the oxidation of CO and volatile chemical compounds occurred over some supported metal catalysts at room temperature[11-12]. These facts imply that these catalysts can be used for disinfections of SARS-CoV, and other microorganisms.

MATERIALS AND METHODS

Preparations of Metal Catalysts Wafers

Ag/Al_2O_3 (Ag 5 wt%) and Cu/Al_2O_3 (Cu 10 wt%) were prepared by an impregnation method. The wet sample was dried at 393 K for 12 h, and then calcined in air at 873 K for 3 h. Before use, the Ag/Al_2O_3, Cu/Al_2O_3 and Al_2O_3 powders were pressed into wafers of ca. 20 mg/cm^2.

Virus Strain Infectivity Testing

SARS-CoV strain CoV-P9 isolated from pharyngeal swab of a probably diagnosed SARS patient in Beijing[8], was passaged on Vero cells. One hundred μL 10^6 $TCID_{50}$/mL viruses was slowly absorbed onto the surfaces of the prepared Ag/Al_2O_3, Cu/Al_2O_3 wafers as well as the support material as a control and stayed at room temperature for 5 and 20 min, respectively. The surfaces of wafers were carefully washed with 100 μL Eagle's medium. After a short spinning at 3 000 rpm, the supernatants were inoculated onto Vero cells in a 96-well plate, meanwhile, 100 μL untreated 10^6 $TCID_{50}$/mL viruses was also infected with the cells as controls. The cytopathic effect (CPE) was monitored after 48 h incubation at 37℃. Viral $TCID_{50}$ was calculated according to Kaerber's protocol.

One hundred μL 10^6 PFU/mL recombinant baculoviruses expressing hamster's PrP protein[13] was exposed and eluted from the surfaces of the wafers as described above, and inoculated onto the insect cell line Sf9 in 25 cm^2 flasks, maintained in SFM medium (Gibco) containing 50 U/mL penicillin and 50 μg/mL streptomycin.

Western Blot

The insect cells infected with the recombinant baculoviruses were harvested and 15 μg crude extracts of Sf9 cells was separated on 12% SDS-olyacrylamide gels (SDS-PAGE) and transferred onto nitrocellulose membranes by electroblotting. Western blot was carried out with PrP specific monoclonal antibody 3F4 (Dako) for 2 h at room temperature and the proteins were visualized with horseradish peroxidase (HRP)-conjuncted anti-mouse antibody (Santa Cruz) according to the protocol described previously[13]. Quantitative analyses of the immunoblot images were carried out using computer-assisted software Image TotalTech (Pharmacia). Briefly, the image of immunoblot was scanned with Typhoon (Pharmacia) and digitalized, saved as TIF format. The values of each target blot were evaluated and balanced with that of a genetic engineering expressed hamster's PrP protein (HaPrP)[13], which was used as a control standard.

Bacterial Strain Inactivation Testing

To see the potential killing effect on prokaryotic cells, *E. coli* strain DH5α containing plasmid pFastBacH10b with ampicillin resistant gene was cultured in LB liquid medium. Roughly 10^6 cells in 100 μL volume were slowly dropped onto the surfaces of wafers for 5 and 20 min and eluted with 500 μL PBS. The elution was spread onto LB agar plates containing 100 μg/mL ampicillin and cultured at 37℃ for 24 h.

Electronic Microscopy Assay

E. coli elution was centrifuged at 10 000 rpm for 5 min and the pellets were resuspended in 50 μL PBS. Ten μL aliquot was absorbed onto 400-mesh carbon-coated cuprum grids for 1 minute. After excess fluid was drained with filter paper, the grids were stained with sodium phosphotungstate, pH 7.5 for about 1 minute. The presence of bacteria was observed with an electron microscope (Philips Tecnai 12) at 80 KV ×7000-12 000.

RESULTS

After exposed to the surfaces of catalyst wafers for 5 min, the infectivity of SARS-CoV elutions on the monolayer Vero cells dropped down to a very low level ($10^{0.5}$ for Ag/Al_2O_3 and $10^{0.75}$ for Cu/Al_2O_3, Table 1). No detectable CPE was observed in the cultured cells inoculated with the elution from that exposed for 20 min (Table 1), whereas the supernatants from the surfaces of support materials induced the typical CPE, revealing a high infectivity ($10^{5.75}$) that was comparable with the result of virus controls. It indicates that exposure of SARS-CoV on

the surfaces of metal catalysts removes viral infectivity *in vitro* efficiently and quickly.

TABLE 1

Decrease of SARS-CoV Infectivity on Vero Cells After Exposed on the Surfaces of the Metal Catalyst Wafers

	Ag/Al_2O_3	Cu/Al_2O_3	Al_2O_3[1]	Virus Control[2]	Cell Control[3]
5 min	$10^{0.5}$	$10^{0.75}$	$10^{5.75}$	$10^{6.25}$	-
20 min		-	$10^{5.75}$	$10^{6.5}$	-

Note.1: Al_2O_3 as the support material in wafer, 2: challenged with 100 μL 10^6 TCID 50/Ml SARS-CoV, 3: Vero cells without virus challenge, -: without detectable CPE.

To address whether exposure to the surfaces of Ag/Al_2O_3 wafers influenced DNA viruses, a recombinant baculovirus was introduced in the tests. No distinct CPE could be found in Sf9 cells after 72 h incubation at 27℃ using the elution of Ag/Al_2O_3 wafer exposed for 5 and 20 min, respectively (data not shown). Western blot assay showed specific reactive bands at the expected position in the lysates of the cells infected with the elution exposed to the surface of the support material (Fig. 1, lanes 5 and 6). However, only slight reactive bands were detected in the lysates of the cells infected with the elution exposed to the surfaces of Ag/Al_2O_3 wafers for 5 min (Fig. 1, lanes 7 and 8), and no band was found in that for 20 min (Fig. 1, lanes 9 and 10). Quantitative analyses revealed that the expression of HaPrP reduced to about 21.8% in the preparation exposed for 5 min, compared with that exposed on the support material.

No colony was identified in the plates inoculated with the elution treated by Ag/Al_2O_3 and Cu/Al_2O_3 wafers for 5 and 20 min, whereas countless colonies were seen in the plates inoculated with the elution from the support materials as controls. Electronic microscopy observations showed that the bacteria in the elution from the control wafer possessed typical figures with intact cell bodies and detectable fibrils (Fig. 2a). After treatment with Ag/Al_2O_3 and Cu/Al_2O_3 wafers for 5 min, the bacterial cell body looked to be lysed, the cell membranes seemed to be cracked and the cytoplasm seemed to be effluent from cell bodies (Fig. 2b), although the bacterial shapes could be seen. After exposure for 20 min, only debris and chips of cells were observed in the elution (Fig. 2c). Compared with the figures of bacilli from the control wafer with a relevant geometric shape and an electronic density, the bacilli exposed for 20 min have lost their geometric shapes. Some of them had visible outlines of cells, but the whole cell bodies were almost electronic transparent and looked like an empty sac with thin membranes, indicating the loss of cytoplasm (Fig. 2c).

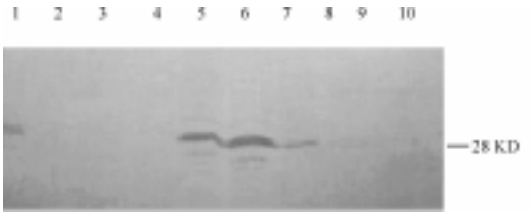

FIG. 1. Expressions of HaPrP proteins in the cell lysates infected with recombinant baculoviruses exposed on the surfaces of Ag/Al_2O_3 wafers. Lane 1: purified HaPrP; lane 2: Sf9 cell; lanes 3 and 4: Sf9 cell with wild type baculovirus; lanes 5 and 6: Sf9 cell with the recombinant baculovirus on control wafer; lanes 7 and 8: Sf9 cell with the recombinant baculovirus on catalyst wafer for 5min; lanes 9 and 10: Sf9 cell with the recombinant baculovirus on catalyst wafer for 20 min. Molecular mass marker is indicated to the right.

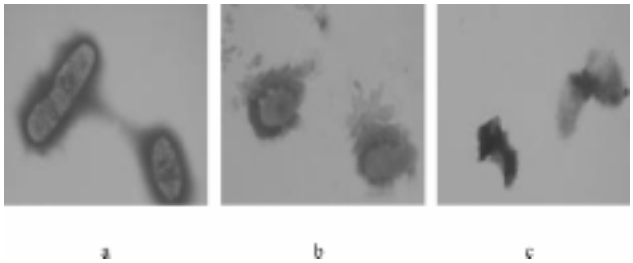

FIG. 2. Electronic microscopy assays of *E. coli* exposed on the surfaces of Ag/Al_2O_3 wafers. a. on control wafer, b. exposed for 5 min, c. exposed for 20 min. (×4900).

DISCUSSION

The transmission of SARS coronavirus is believed to be through droplets and fomites, as well as airborne. Air disinfection plays important role in interdicting the transmission pathway for SARS and other respiratory infectious diseases. Surface science and catalysis studies showed that metal catalysts could be used for air-disinfection. Exposures of organic chemical compounds to the surfaces of some metal catalysts at room temperature could result in decomposition, probably due to the oxidation by the dissociated active oxygen atoms on the metal surfaces[9,11]. Here, we reported that exposure to the surfaces of Ag/Al_2O_3 and Cu/Al_2O_3 could destroy the replication and propagation abilities of SARS-CoV, baculovirus and *E. coli* in their respectively optimal culturing situations *in vitro*. Although the catalysing procedures on these microbes on Ag/Al_2O_3 and Cu/Al_2O_3 surfaces still remain unknown, the oxidation effects on some organic components on the surfaces of microbes may have a contribution. In our study elimination of SARS-CoV infectivity was only obtained when the loading viruses on the catalyst surfaces were exposed to air, and the buffer in which suspended viruses filtered into the support materials. We found that viral infectivity in Vero cells was almost unchanged when virus droplet was large on the surfaces of catalyst wafers (data not shown) that might block off the interaction of metal catalysts with air, leading to impeding usage of oxygen molecules in air. It is suggested that virus-killing capacities on metal catalyst surfaces depend on the efficient usage of oxygen in air.

Electronic microscopy analyses revealed clear extravasation of cytoplasm from *E. coli* bodies after treatment on the surfaces of metal wafers. One might speculate that cell membranes could be perforated by oxidation effects on some organic components. Although it is hard to get morphological evidence of viruses after exposure and destroy of viral infectivity may go through different pathways from bacteria, ubiquitous oxidation effects on viral proteins or structure may result in the loss of replicating capacity of DNA or RNA viruses, and capsulated or naked viruses.

Numerous micropathogens could be respiratorily transmissed through air, droplets or fomites, *etc*. During the last few decades, central air-condition or air-exchange systems have been reported as a pathway to transmit infectious agents. Although using effective filters in air-exchange facilities can block the potential microbes, it is unable to kill them or rule out their infectivity. Efficiently killing viruses and bacteria on the surfaces of metal catalysts has a promising potential for air-disinfection in hospitals, communities, and households.

ACKNOWLEDGEMENTS

We thank Mr. Tong-Xing ZHAO and Ms. Hui-Ying JIANG from Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, for their excellent technique support for electronic microscopy assays. We are indebted to Mr. Wei-Dong YIN from Beijing Sinovac Biotech Co., Ltd. for the helpful discussion.

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(*Received January* 18, 2004 *Accepted September* 23, 2004)