Prevention the spread of norovirus infection on airborne route by using Plasma Assisted Catalytic Technology (PACT) Device.

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Running Head: Norovirus sterilization method by using PACT device

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ABSTRACT.

Zoonosis becomes a popular word. Highly pathogenic influenza virus (HPI), Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) recently occurred at around Africa, Meddle-East and South-East Asia area, whose virus is classified as airborne. Sterilization capability was investigated by using chemical reactor of PACT device. Test on airborne infection was carried out by Feline Calicivirus Vaccine (FCV) strain F9, which is also surrogated human norovirus. It was found that PACT device could sterilize instantly FCV when passing through the plasma space of PACT device. Sterilization rate may be more than 99.99% (below the detection limit). This result may be available to sterilize various virus including human norovirus and airborne-infectious microorganisms.

*Keyword:* FCV, pathogens, plasma, sterilization, zoonosis
Airborne infectious diseases, such as SARS, *Mycobacterium tuberculosis*, and highly pathogenic avian influenza, are threats to the health management of humans. In health care institutions, infected persons visit and move, which not only increases the change of infection for many patients and health care workers but also leads infected immunocompromised persons to a serve state and death [16]. Infection with *Mycobacterium tuberculosis*, measles virus, and varicella virus spreads through the cough and sneeze of infected persons, in which scattered droplets are dried and form dried droplet nuclei alone or adhering to particles in the air, and infection occurs by inhalation of them. When droplet nuclei are particles of less than 5μm diameter, they can float in the space for a prolonged time or widely spread [1, 16]. Airborne infection is partially dependent on the concentration of infectious pathogen in the air [8]. Vaporized material from infected person’s vomit may affect airborne infection. The typical pathogen transmitted by airborne infection is norovirus. Cases of infection through an air conditioner 1~2 days after processing vomit from norovirus infected persons have been reported [3, 7, 15].

Therefore, comprehensive removal of airborne infectious pathogen is important for the health care of humans and animals sharing a space, and development of an effective ventilation method is necessary [2]. Although filters of heating, ventilation, and air conditioning (HVAC) systems are capable of controlling pathogens, they do not effectively sterilize pathogens and it is possible that pathogens are released from the filter [10]. Accordingly, Plasma Assisted Catalytic Technology (PACT) device is a novel chemical reactor, which can decompose various hazardous gases like Volatile Organic Compounds (VOC) [13, 14]. PACT concept shows off the synergistic
effects between plasma excitation or catalytic activation [6, 14, 17, 18]. Moreover, PACT device has functions of ozone including oxygen radicals [5, 13]. PACT device may be expected to sterilize airborne virus more efficiently, compared to the
conventional sterilization method.

FCV-F9 (VR-782, 40 nm diameter) virus used in the study is a vaccine strain. Crandell Rees feline kidney (CRFK) cells were used for culture of FCV and measure the virus. Cells were cultured in Eagle MEM (Nissui, Tokyo, Japan) containing 5% fetal bovine serum (Nichirei Bioscience Inc, Tokyo, Japan), 0.3% Tryptose Phosphate Broth (BD Biosciences, Sparks, MD, U.S.A.) and antibiotics.

The configuration of PACT device is shown in Fig.1. All of the aerosol gas could be passed through plasma space, which is a gap of 1mm. High voltage (10kVp-p, 10kHz) is applied to this gap to form the plasma excitation zone by stable barrier discharge. Test method is shown in Fig.1D. The FCV F9 strain was sprayed, using nebulizer (Omron, Kyoto, Japan) for 5 min, in a chamber with approximate 15 l volume placed in a safety chamber. After virus passes through the plasma space of PACT device, the residual virus were captured by a gelatin film filter set in an air sampler (MD8 Airscan: Sartorius, Göttingen, German). The captured virus was suspended with 2.5 ml of 37°C culture medium and the recovered virus was measured by the tissue culture infectious dose 50% (TCID50) method. To optimize, the test was investigated by changing the air flow rate, applied voltage to PACT device, and the amount of FCV F9 strain. So this is a flow of contaminated air though a fixture which opens into the PACT device then passing through the gelatin filter and ending in an enclosed space.

The test was carried out following the dependency on sterilization effects
between flow rate and power of the PACT device. To clarify the relationship between
flow rate and sterilization, FCV sprayed for 5 min. was captured by a gelatin film filter
at 2 ~ 8m$^3$/min by 134W load on the PACT device. Without the PACT device
operational, the mean titers of virus recovered at 2, 4 and 8m$^3$/min were $2.4 \times 10^3$,
$4.3 \times 10^4$ and $3.3 \times 10^4$ TCID$_{50}$/100$\mu$l respectively (Fig. 2A). No difference was found in
the amount of the virus recovered at 4m$^3$/min or higher, it was nearly 10 times lower at
2m$^3$/min. On PACT device operation, the recovered virus titer at 2 m$^3$/min was lower
than the detection limit. The mean titers at 4 and 8 m$^3$/min were $2.7 \times 10^1$ and $6.3 \times 10^1$
TCID$_{50}$/100$\mu$l, respectively, the sterilization effects of the PACT device were $2.4 \times 10^3$,
$1.58 \times 10^3$, and $5.21 \times 10^2$ TCID$_{50}$/100$\mu$l at 2, 4, and 8m$^3$/min, respectively (Fig. 2B). It
was clearly found that PACT device can sterilize instantly through the plasma zone and
the efficiency rate depends on the flow rate of negative pressure flow, as aerosol
including FCV F9 strain.

To clarify the sterilization dependency upon the input power to PACT, the
captured virus on gelatin film filter was measured by changing the input power between
100 ~ 167W. The mean recovered virus titer was $2.2 \times 10^4$ TCID$_{50}$/100$\mu$l at 2m$^3$/min. The
recovered virus titer was lower than detection limit in the case of 167W, and the mean
titers at 134 and 100 W were $1.2 \times 10^1$, $9.7 \times 10^1$ TCID$_{50}$/100$\mu$l, respectively (Fig. 3A).
Reduction rate of virus titer was calculated from the obtained data in Fig. 3A. It was
clarified that its rate increased as the input power increased to the PACT device.

PACT was developed as an air cleaner and it removes toxic substances in
cigarette smoke. It has recently been utilized to inactivate bio-aerosols employing
plasma technology [5]. We investigated whether PACT has a virucidal effect against an
airborne-infectious virus. Aerosolized FCV was inactivated by being passed through
PACT and the FCV titer was reduced to below the detection limit depending on
conditions.

In this paper, the sterilization of virus means including remove, and inactivation.
There exist various methods to sterilize virus, whose main mechanism is plasma,
photo-catalyst, ozone including oxygen radicals and so on [5, 9]. When the peptide
connected protein of cell wall will be damaged like as break or displacement, bacteria or
fungi may be sterilized. Ito reported that O-related radicals of non-equilibrium
atmospheric pressure plasma are important mechanism for the inactivation of fungus
spores [4]. Murray reported that plasma is dominant factor of sterilizing various type of
virus [11]. It has been utilized to sterilize bio-aerosols by applying plasma technology [9,
18]. The mechanism of bacterial sterilization by plasma is caused to damage bacterial
cell wall and to leakage the cytoplasma, and reactive oxygen enters cell through the
injured site of pathogen including bacteria, fungi, and viruses [11, 19].

In the case of PACT device, its plasma radiation is formed non-equilibrium, so
plasma is higher temperature of electron (~10,000eV) and lower temperature of gas
(~1,000K). Increasing temperature (~98°C) of PACT device on operation may not affect
various chemical reactions. PACT device may be expected to sterilize instantly virus,
bacteria, and fungi at both hospitals and clinics, that can be a case of nosocomial
infection. Nishimura evaluated some commercially available air cleaners and reported
they are almost ineffective to inactivate air-borne influenza virus in real life space
(re-circulating ambient) [12]. Our results of PACT carried out using a single pass
ambient air controlled experiments. In addition, more studies need to evaluate the
virucidal effect of PACT device in real life space. We need to scale up the experiment to
replicate a living space, and measure the relationship between flow and efficiency in a
larger plenum chamber.

In conclusion, PACT device can sterilize FCV F9 instantly when passing through
plasma space formed between electrode’s gap. Sterilization rate depends upon both
the gas flow rate and the input power to PACT device. FCV F9 virus is classified in
positive strand RNA without envelope virus, these test results are useful for same group
like as human norovirus. Various type virus could be sterilized mainly by plasma
dominant mechanism and similar virus like SARS, MERS and influenza virus, which
has a single strand RNA with envelope virus could be expected to be sterilized.
Considering the molecular size, PACT may apply to the sterilization against bacteria
and fungi as well. According to the above results, PACT can be developed especially for
Pathogen Buster, which maybe a countermeasure for zoonosis.

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Fig. 1. Schematic representation of the experimental devices used in this study.

A: Outer view of PACT device, B: The details of PACT device, C: Plasma radiation when PACT device is operated, D: Experimental devices for sterilization.

Fig. 2 Effect of air speed and virucidal effect of PACT treatment.

A: Determination of captured virus titer in different air speed conditions. B:

Determination of virucidal effect of PACT.

Fig. 3 Effect of voltage and virucidal effect of PACT treatment.

A: Determination of captured virus titer in different voltage conditions. B:

Determination of virucidal effect of PACT.
Fig. 1

A. PACT Device

B. Diagram of PACT components:
- Air Flow
- Support Plate (Inner)
- Support Plate (Outer)
- Spacer for Gap
- Dielectric Material
- Electrode (High voltage)
- Electrode (Earth)

C. Image of PACT device in use

D. Schematic of PACT system:
- Air Sampler
- Gelatin Membrane
- Chamber
- Nebulizer

PACT Device
Fig. 2

A

Virus titer

\[ \begin{align*}
\text{PACT} & \quad 2m^3/h \quad \text{PACT} & \quad 4m^3/h \quad \text{PACT} & \quad 8m^3/h \\
- & \quad + & \quad - & \quad + & \quad - & \quad +
\end{align*} \]

B

Reduction of virus titer

\[ \begin{align*}
PACT (m^3/h) & \quad 2 \quad 4 \quad 8 \\
10^0 & \quad 10^1 & \quad 10^2 & \quad 10^3 & \quad 10^4
\end{align*} \]
Fig. 3

**A**

![Graph A](image)

**B**

![Graph B](image)