Development of novel air purification technology using ions generated by discharge plasma (ii) inactivation of influenza virus in air

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ABSTRACT
Novel indoor air purification technology using ions generated by discharge plasma has been developed. The effect of ions on airborne influenza virus has been investigated by the plaque method using MDCK cells and the hemagglutination test. It has been discovered that these ions can inactivate influenza virus in air world first. The infection rate of influenza virus to MDCK cells has been drastically reduced with the use of ions generated by the developed device. Furthermore the efficacy tests of these ions for polio virus and coxsackie virus have been performed. The significant decreases of these viruses with the operation of the device have been observed similarly.

INDEX TERMS
Airborne virus, Air quality, Indoor air, Ions, Discharge plasma

INTRODUCTION
Vaccine and antibiotic for prevention against virus infection have been studied intensively. Particularly, influenza is the most problem in the world. Research and development of technology for prevention against the airborne virus infection is very significant. We have developed novel indoor air purification technology using ions, of which physical background and fundamental properties are reported another paper in this conference. The purpose of this paper is to report the effect of ions on airborne Influenza virus.

METHODS
The structure of the ion generation device
Figure 1 shows schematic cross sectional diagram of the ion generation device. The developed ion generation device consists of ceramic dielectric plate and attached high voltage applied and ground electrodes. With the application of AC voltage between the electrodes, discharge plasma occurs at the surface of the ceramic plate. Positively charged ions and negatively charged ions were generated at atmospheric pressure. The concentration of ozone has been confirmed to be less than 0.005 ppm at vicinity of the device.

The measuring method of the generated ions
The ion densities were examined by measuring electrical conductivity of air by means of a

double co-cylinder shaped detector, called a Gerdien condensor. The chemical compositions of the generated ions were examined by Time of Flight mass spectroscopy.

Test Apparatus

Figure 2 shows the schematic diagram of the test apparatus. The test device was attached and secured to one end of an acrylic cylindrical container with a length of 200 mm and inner diameter of 55 mm, and this assembly was placed in a polyvinyl chloride cubical container. A virus liquid atomizer was attached on one side of the container, while an apparatus for recovering the virus liquid was attached to the other side. This entire system was then placed inside and sealed within another, lager polyvinyl chloride container.

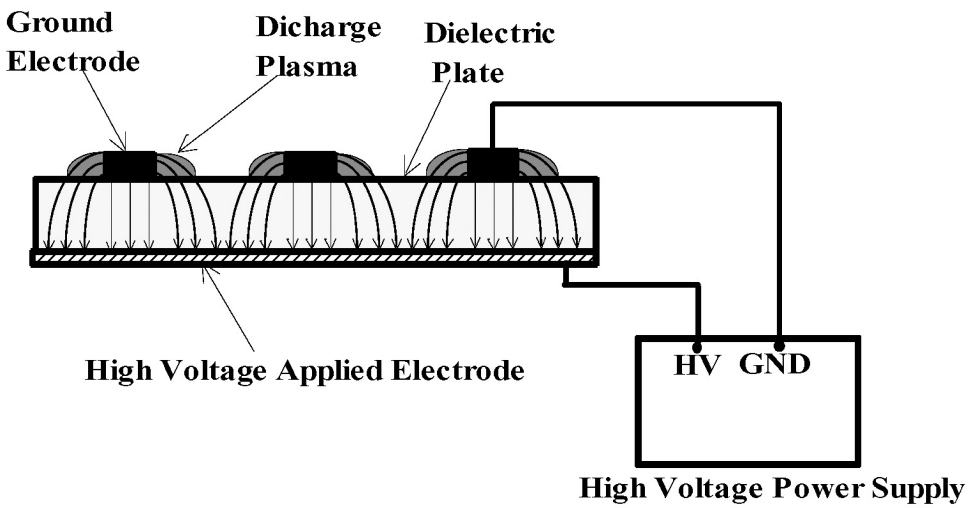


Fig. 1 Schematic cross sectional diagram of ions generation device

Test Method

In the virus test, *Influenza virus A(H1N1) A/PR8/34, Polio virus I Sabin (Lsc. 2ab),* and *Coxsackie virus B6* have been used as test virus. 10 ml of the test virus liquid was placed in a glass atomizer and the atomizer connected to one end of the test apparatus. A glass impinger containing 10 ml of phosphate buffered saline (PBS(-)) was connected to the other end of the test apparatus. The velocity of compressed air form an air compressor was adjusted to air velocity 4m/s in the cylindrical container, and the test virus was atomized from a spraying nozzle. The atomization volume was set to 3.0 ml (atomization flow rate of 0.1 ml/min x atomization time of 30min). At this time, the state when the power of the test device was not switched on was set as the control. A comparison was made between operating the test device with the generated positively and negatively charged ions densities set to 200 thousands, 100 thousands, 50 thousands, and 5 thousands, and the control. The impinger was made to aspirate and capture the air inside the test apparatus for 30 minutes at an aspiration rate of 10 l/min.

Virus Quantification Method

Influenza viruses were measured by the hemagglutination reaction (HA) and the plaque method using MDCK cells and by using PBS(-) into which the air in the test apparatus had been aspirated and captured with the impinger as the test liquid.

The PBS(-) captured by aspirating the air contained viruses inside the test apparatus with the impinger was used as the test liquid, and Polio virus and Coxsackie virus was quantified with the plaque method using HeLa cells.

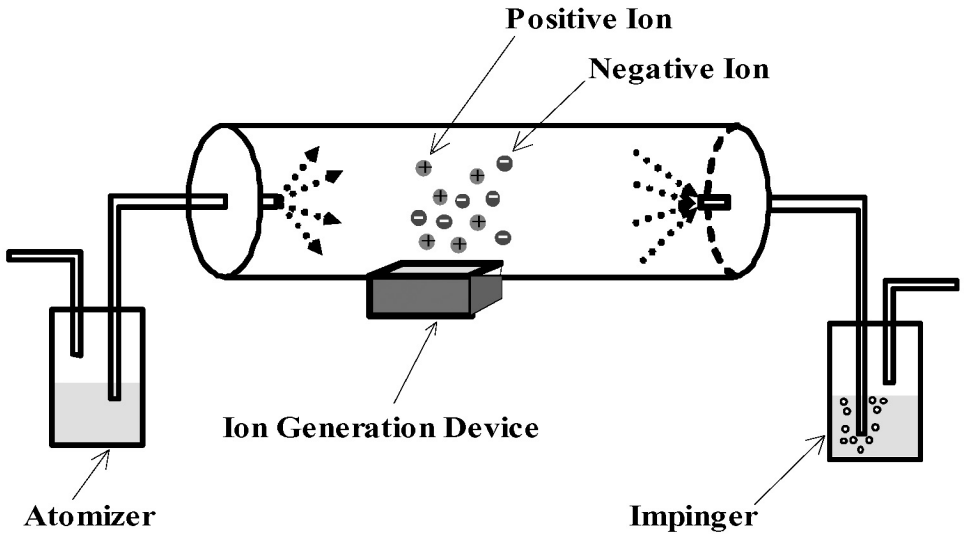


Fig.2 Schematic diagram of the test apparatus

RESULTS

Reduction of the infection capacity of airborne Influenza viruses

Figure 3 shows plaque forming rate of airborne Influenza viruses measured by the plaque method using madin-darby canine kidney (MDCK) cells. When Influenza viruses were not exposed to ions, Influenza virus plaque forming units were 1,500• 2,800 PFU/300L-Air. On the other hand, when Influenza viruses were exposed to ions with density 5,000, 50,000, 100,0000 and 200,000 counts/cm^3, it has been found that Influenza virus plaque forming units were greatly reduced to 110• 120, 62• 80, 40• 60 and <10 PFU/300L-Air respectively. In the result, it has been confirmed that the exposure to ions reduced infection capacity of Influenza viruses. To distinguish the effect of ozone generated by the device, we performed similar test with ozone of 0.005 ppm and without ions. As a result, exposure to ozone of 0.005ppm has not reduced infection capacity of Influenza virus.

Figure 4 shows efficacy evaluation against Influenza viruses test of viral infection in MDCK cells. Cells inoculated with Influenza viruses that were exposed to ions have been not infected. Cells inoculated with Influenza viruses that were not exposed to ions have been extincted by viruses infection.

Figure 5 shows the hemagglutination reaction (HA) test of Influenza viruses. Red blood corpuscle (RBC) inoculated with Influenza viruses that was not exposed to ions has been found the hemagglutination reaction. RBC inoculated with Influenza viruses that was exposed to ions has not been found the hemagglutination reaction.

Reduction of the infection capacity of airborne Polio virus and Coxsackie virus

Figure 6 shows plaque forming rate of airborne Polio viruses measured by the plaque method using HeLa cells. When Polio viruses were not exposed to ions, plaque forming units of Polio viruses were 5,600• 6,800 PFU/300L-Air. When Polio viruses were exposed to ions with density 50,000, 100,0000 and 200,000 counts/cm^3, it has been found that plaque forming units of Polio virus were greatly reduced to 42• 54, 30• 36 and 20• 30 PFU/300L-Air respectively. In the result, it has been confirmed that the exposure to ions reduced infection capacity of Polio virus.

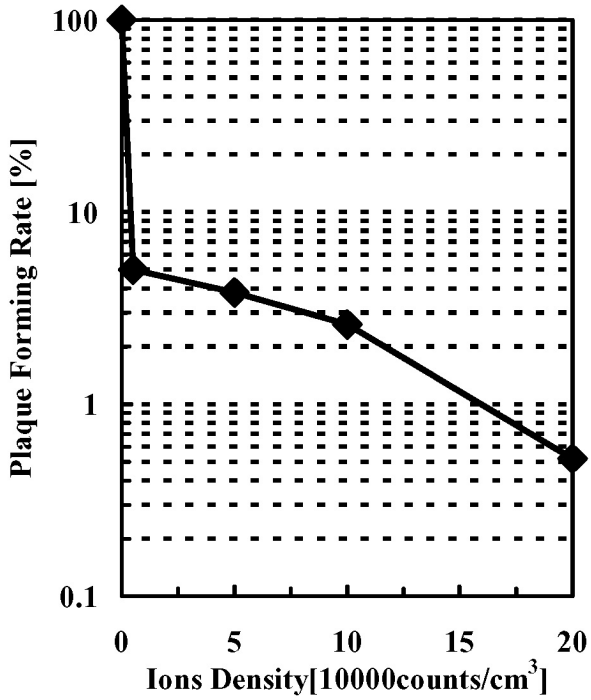
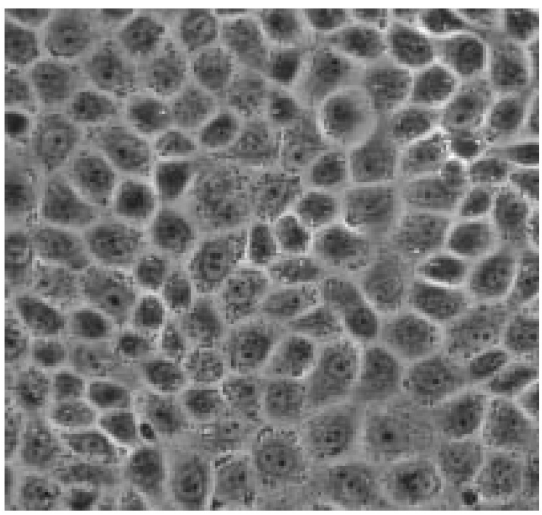
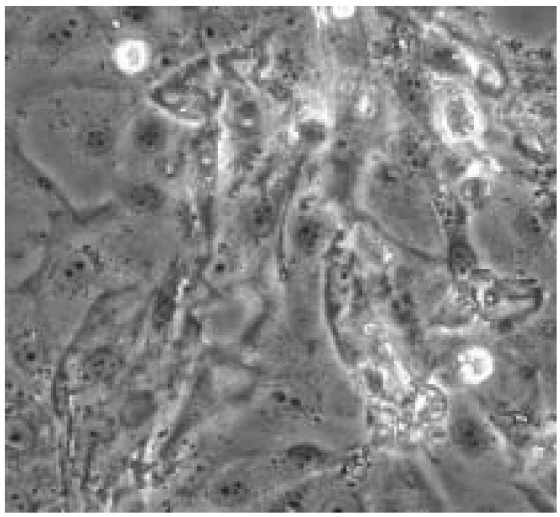


Fig.3 Influenza virus plaque forming rate by the plaque method using MDCK cells



Cells inoculated with influenza virus that was exposed to ions [No virus infection]



Cells inoculated with influenza virus that was not exposed to ions [Cells extincted by virus infection]

Fig.4 Efficacy evaluation against influenza virus test of viral infection in madin-darby canine kidney (MDCK) cells

Figure 7 shows plaque forming rate of airborne Coxsackie viruses measured by the plaque method using HeLa cells. When Coxsackie viruses were not exposed to ions, plaque forming units of Coxsackie viruses were 2,200 to 3,000 PFU/300L-Air. When Coxsackie viruses were exposed to ions with density 50,000, 100,0000 and 200,000 counts/cm^3, it has been found that plaque forming units of Coxsackie viruses were greatly reduced to 75• 90, 48• 80 and 12• • 38 PFU/300L-Air respectively. In the result, it has been confirmed that the exposure to ions reduced infection capacity of Coxsackie viruses.

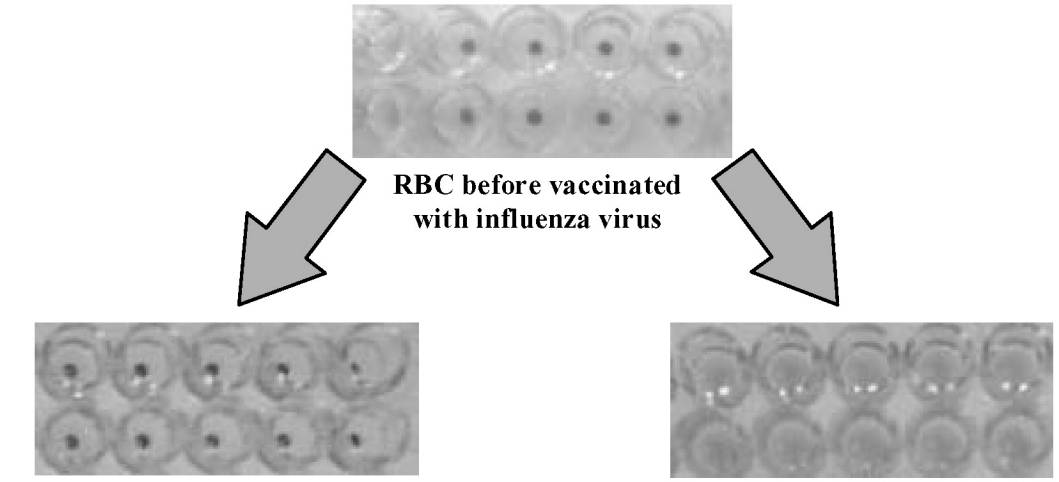


Fig.5 Hemagglutination reaction (HA) test
Observation the mode of red blood corpuscle (RBC) vaccinated with influenza virus

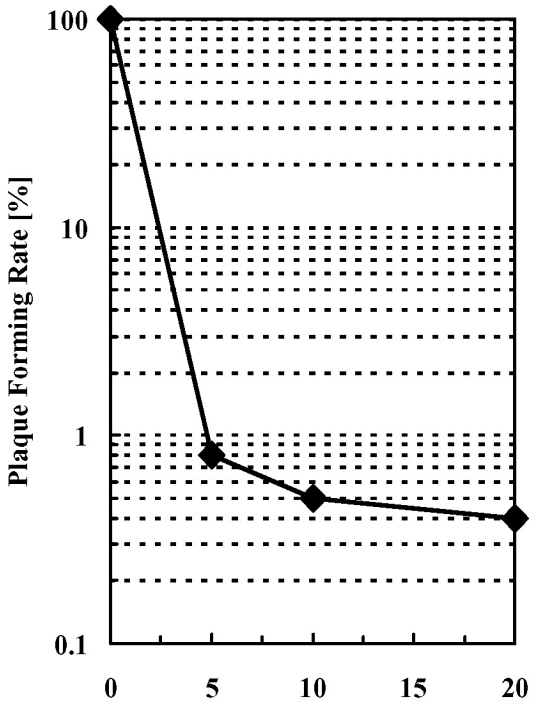


Fig.6 Polio virus plaque forming rate by the plaque method using HeLa cells

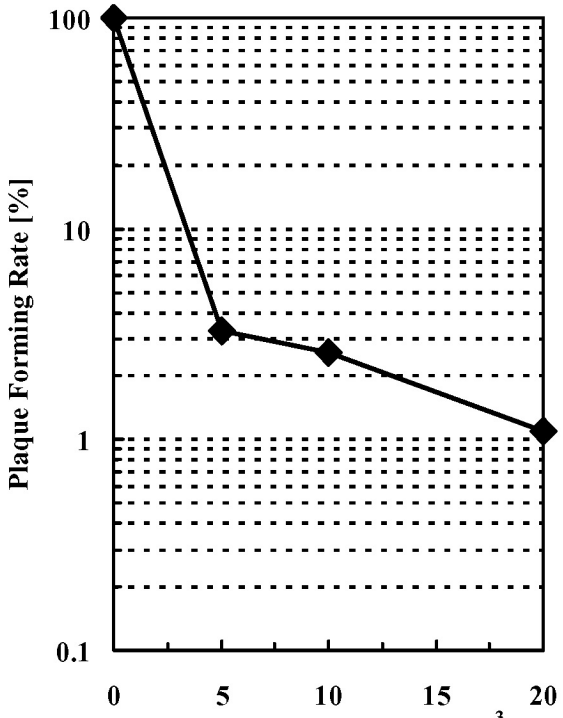


Fig.7 Coxsackie virus plaque forming rate by the plaque method using HeLa cells

DISCUSSION

These test results show that the ions generated by the developed ion generation device have inactivated hemagglutinin, a spike-shaped protein located on the outer surface of the viruses, thereby altering the make-up of the protein molecule. Figure 8 shows the mechanism for reduction of infection capacity of viruses. As a possible explanation for the obtained the infectious capacity reduction effects of the ions generated by discharge plasma, it is

considered as follows. When the generated ions collide with viruses, positively charged ion $H^+(H_2O)_m$ and negatively charged ion $O_2^-(H_2O)_n$ react on the viruses and generate some active species, for example HO_2, H_2O_2 and OH radicals. These active species probably break the protein located on the surface of the viruses. As a result the ions have reduction properties of the infection capacity of viruses. We are now investigating the mechanism of these characteristics with atom-scale techniques.

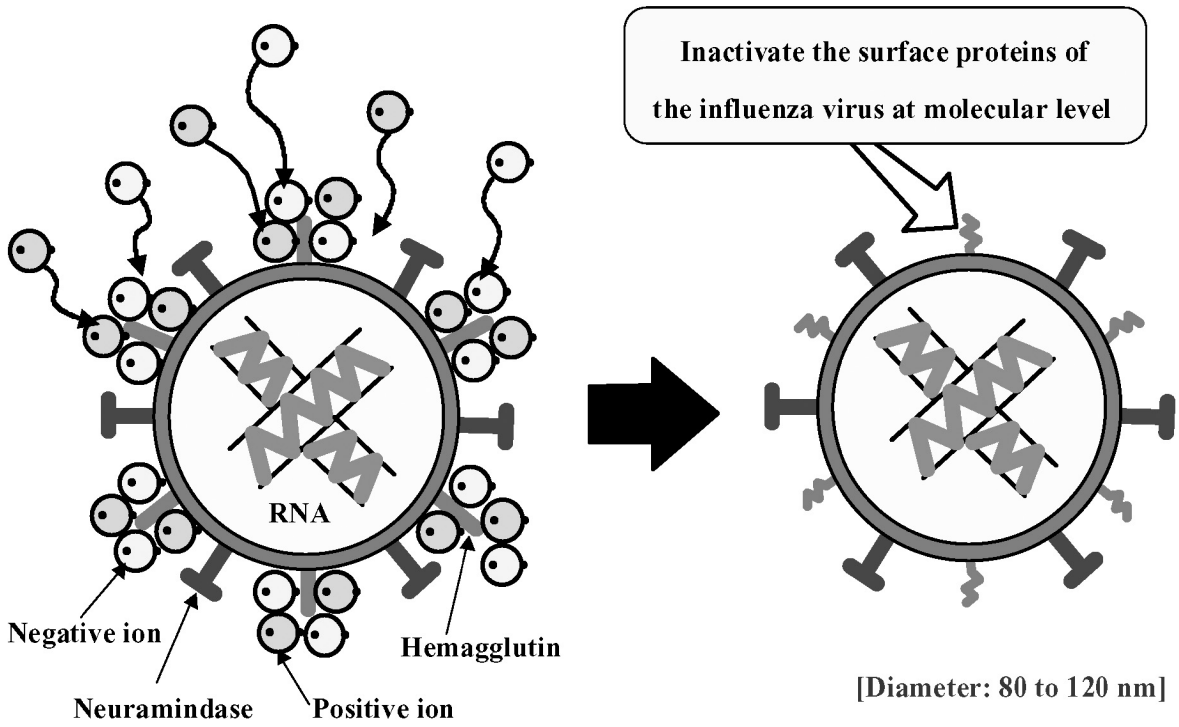


Fig.8 Mechanism for reduction of infection capacity from viruses

CONCLUSION AND IMPLICATIONS

Novel indoor air purification technology using ions generated by discharge plasma has been developed. We have discovered that infection capacity of airborne viruses exposed to positively and negatively charged ions has been greatly reduced. This discovery is greatly expected as the novel infectious prevention method in indoor air.

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