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Evaluation of a bipolar ionization device in inactivation of antimicrobial-resistant bacteria, yeast, *Aspergillus* spp. and human coronavirus

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SUMMARY

Background: The efficacy of bipolar ionization in the healthcare setting has yet to be proven. A major limitation of studies sponsored by industry has been the assessment of efficiency within test chambers in which ozone levels are not adequately controlled.
Aim: To assess the effectiveness of bipolar ionization against antimicrobial-resistant bacteria, fungi and human coronavirus within a controlled test chamber designed to mitigate the effect of ozone.
Methods: Bacteria- and fungi-inoculated gauze pads, and human coronavirus 229E-inoculated stainless steel plates were placed within the vicinity of the AIO-2 bipolar ionizer and left at room temperature (2 h for coronavirus and 4 h for bacteria and fungi).
Findings: Four hours of exposure to bipolar ionization showed a 1.23–4.76 log reduction, corresponding to a 94.2–>99.9% colony-forming units/gauze reduction, in *Clostridioides difficile*, *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*, meticillin-resistant *Staphylococcus aureus* and multi-drug-resistant *S. aureus*. A 1.2 log 50% tissue culture infectious dose reduction in human coronavirus was observed after 2 h.
Conclusion: The assessment of bipolar ionization systems merits further investigation as an infection control measure.

Introduction

Since the occurrence of the coronavirus disease 2019 (COVID-19) global pandemic, there has been a growing market for air purification and reduction of surface contamination using air ionization devices [1]. The antimicrobial effectiveness of ions has long been controversial. A review by Krueger and Reed concluded that negative and positive ions inhibit growth of micro-organisms [2]. A study focusing on static charges on fomitic surfaces and deposition of bacteria showed that bipolar ionization resulted in a reduction of bacterial deposition [3]. In a trial conducted in an intensive care unit, Kerr *et al.* found that negative air ionizers were associated with a significant decrease in acinetobacter infections as well as patient colonization [4]. In a recent real-world hospital setting, the implementation of a technology using charged particles reduced healthcare-associated infections (HAIs) by 45% [5].

Of the various technologies claiming to deactivate bacteria, viruses and fungi, variants of bipolar ionization technology (needle-point, corona discharge, plasma cluster etc.) have enjoyed renewed commercial popularity as a result of the COVID-19 pandemic. In bipolar ionization, positive (H^+) and negative (O^{2-}) ions are generated when water molecules are

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exposed to high-voltage electrodes. The mechanism(s) associated with the biocidal effect of positive and negative ions have not been clearly established. The purported mechanism of the inactivation of micro-organisms and viruses is the clustering of these ions around viruses and micro-organisms, resulting in the formation of OH radicals, which remove hydrogen, and the formation of water vapour, leading to inactivation [6].

While droplet and airborne transmission are considered to be the main routes of exposure in the ongoing COVID-19 pandemic, disinfection of contaminated or potentially contaminated surfaces is also one of the strategies for controlling COVID-19, as it has been shown that severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) can remain viable on different surfaces from hours to a few days [7]. In this study, an independent evaluation of a bipolar ionization technology device against antimicrobial-resistant bacteria, *Candida albicans*, *Aspergillus fumigatus* and human coronavirus was performed in a controlled laboratory environment, in which ozone and the concentration of ions were not allowed to accumulate in an enclosed environment. The objective was to assess the potential utility of this technology within a healthcare environment as an adjunct to existing protocols for minimizing HAIs.

Methods

Bacteria, fungi and virus tested

The organisms tested were ATCC strains of meticillin-resistant *Staphylococcus aureus* (MRSA, ATCC 43300), *Clostridioides difficile* (ATCC 17857), *C. albicans* (ATCC 10231), *A. fumigatus* (ATCC 13073) and human coronavirus 229E (ATCC VR-740), as well as clinical isolates of multi-drug-resistant *Pseudomonas aeruginosa* (MDRP), multi-drug-resistant *Acinetobacter baumannii* (MDRAB) and *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-KP).

Bipolar ionizer

An H-ION CLUSTER Industrial Air Purifier Module (AIO2) (Sudo Premium Engineering, Seoul, Korea) was used in this study.

Preparation of organisms to be tested

MRSA, MDRP, MDRAP and KPC-KP were plated on trypticase soy agar and incubated for 18 h at 35 °C. *C. difficile* was cultured using an anaerobic blood agar plate (Centers for Disease Control and Prevention formulation) with 5% horse blood, and incubated using an AnaeroPak rectangular jar and Anaeropack gas generator (Mitsubishi Gas Chemical, Tokyo, Japan) for 48 h at 35 °C. *A. fumigatus* was inoculated on potato dextrose agar and cultured at room temperature for 1 week. Human coronavirus 229E was propagated for 3—5 days on MRC-5 cells (JCRB No. B0521) in E-MEM supplemented with 10% fetal bovine serum (FBS). Suspensions of bacterial equivalent to McFarland 0.5 were prepared and diluted 1:150 using physiological saline for a concentration of approximately 1.0 x 10^6 colony-forming units (cfu)/mL. For *A. fumigatus*, a spore suspension was prepared and diluted using physiological saline for a spore suspension of approximately 1 x 10^5 spores/mL. Similarly, a suspension of *C. albicans* equivalent to approximately 1.0 x 10^6 cfu/mL was prepared. One millilitre of the prepared bacterial and fungal suspensions was applied to a sterile, 5 cm x 5 cm gauze pad (Kawamoto Corporation, Osaka, Japan) and left at room temperature [8]. Sterile 1.5 cm x 1.5 cm SUS304 (Japanese SIS standard) stainless steel squares were loaded into sterile Petri dishes using sterile forceps. A 4.3 $\log_{10}$ 50% tissue culture infectious dose ($TCID_{50}$)/mL suspension of human coronavirus 229E tissue culture was applied to the surface of the stainless steel squares and left at room temperature [9].

Test environment

Based on several other publications on the evaluation of air purification systems, an acrylic chamber was selected as the test environment [10,11]. Testing was performed within a 240-L acrylic chamber that measured 100 cm (W) x 60 cm (D) x 40 cm (H). The acrylic chamber was placed within a Class II (Type A1) biological safety cabinet (BSC).

As ozone is heavier than air and therefore tends to sink, the inoculated gauze and steel plates were placed on a platform 30 cm higher than the floor of the acrylic chamber. In order to prevent the accumulation of ozone and ions within the acrylic chamber, the acrylic chamber was raised 2 cm above the BSC workspace to pull air out of the chamber. A Model 1200 ozone counter (Dairec, Inc., Kurashiki City, Japan) was placed within the acrylic chamber to monitor ozone levels within the negative pressure environment. The temperature within the test chamber was 21—22 °C and 38—50% relative (Figure 1).

Measuring the effectiveness of bipolar ionization

The bacteria- and fungi-inoculated gauze pads, and the human coronavirus 229E-inoculated stainless steel plates were placed within the vicinity of the AIO-2 bipolar ionizer and left at room temperature for up to 4 h. The distance between the gauze pads or stainless steel plates and the AIO-2 bipolar ionizer was 35 cm. As a control, inoculated gauze pads and stainless steel plates were placed within another acrylic chamber without the AIO-2 ionizer.

After 4 h, bacteria and fungi from each of the gauze pads were extracted by immersion in tubes containing 10 mL of sterile physiological saline and vortexed. Ten-fold serial dilutions of the suspensions were performed using sterile physiological saline, after which 0.1 mL of each of the dilutions was inoculated using a spreader on to either trypticase soy agar, anaerobic blood agar plate with 5% horse blood or potato dextrose agar depending on the organism. Following incubation, colony counts of three replicates were performed to determine the mean colony counts.

As the infectivity of human coronavirus 229E decreases significantly after drying on various surfaces, exposure to bipolar ionization was for 2 h [12]. The inoculated stainless steel squares were then retrieved, immersed immediately in 2 mL of E-MEM without 10% FBS and vortexed, followed by serial 10-fold dilution using E-MEM without 10% FBS. Quantitation of viable human coronavirus 229E was performed using 96-well tissue culture microtitre plates that were seeded with MRC-5 cells. After washing the tissue culture microtitre plates with E-MEM without 10% FBS, 100 μL of the E-MEM used to elute inoculated virus on stainless steel plates was inoculated on to

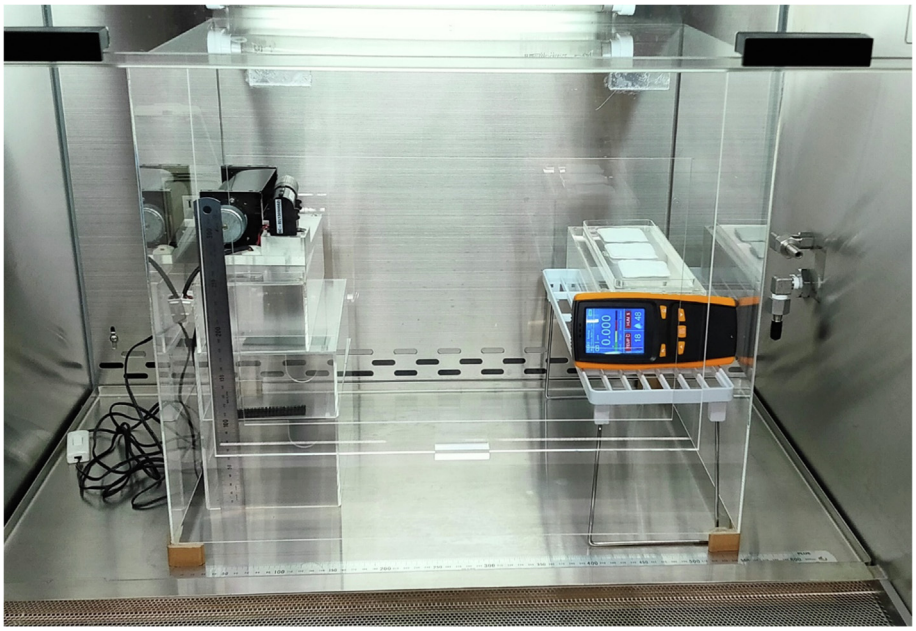

Figure 1. Test environment.

MRC-5 cells. After allowing for absorption for 1 h at 37 °C under 5% CO_2, E-MEM without 10% FBS was removed from the tissue culture microtitre plates, and 100 μL of E-MEM containing 2% FBS, 100 U/mL of penicillin and 100 U/mL streptomycin was dispensed into each well and incubated for 7 days at 37 °C at 5% CO_2. Following incubation, the cell culture media was removed from the microtitre trays, and MRC-5 cells were exposed to 70% ethanol for 20 min. Following fixation, MRC-5 cells were observed for cytopathic effect to determine $TCID_{50}$ using the Behrens–Karber method.

Measurement of the effectiveness of bipolar ionization was performed in triplicate.

Log_{10} cfu/gauze and $TCID_{50}$/mL reduction following exposure to bipolar ionization

Log reduction between controls and post-exposure was calculated using the following equation: Log_{10} (A/B), where A is cfu/mL or $TCID_{50}$/mL after treatment and B is cfu/mL or $TCID_{50}$/mL before treatment [13]. To convert the log reduction to a percentage reduction, % reduction $= (1 - 10^{-L}) \times 100$ was utilized, where L represents log reduction.

Results

As shown in Table I, 4 h of exposure to bipolar ionization showed a 1.23–4.76 log reduction, corresponding to a 94–>99.9% cfu/gauze reduction, in *C. difficile*, KPC-KP, MRSA and MDRP. MDRAB was the only bacterium tested that showed <1 log or 90% cfu/gauze reduction after 4 h. In addition, a <1 log reduction was observed with no significant inactivation of *A. fumigatus* spores. *C. albicans* also showed <1 log reduction, as exposure to bipolar ionization led to an 87.3% reduction in cfu/gauze.

Two hours of exposure to positive and negative ions led to a 1.2 log reduction in human coronavirus 229E, corresponding to a 94.0% $TCID_{50}$ reduction (Table 2).

During the triplicate testing of the organisms, ozone ppm was <0.055 except for one experiment in which the ozone ppm was 0.066. The ozone concentration was monitored continuously during bipolar ionization exposure, and did not show fluctuation as the air within the acrylic chamber was pulled out continuously. Ozone also served as a surrogate for positive and negative ions to ensure that the concentration of ions within the chamber was constant.

Discussion

Since the beginning of the COVID-19 pandemic, a number of hospitals, schools, casinos and sports arenas have implemented variants of bipolar ionization as a technology to disinfect air and surfaces [14]. However, one manufacturer is currently the subject of a class action lawsuit which claims that the manufacturer falsely claimed that its technology was effective against SARS-CoV-2 [15]. The suit cites Boeing's technical assessment of bipolar ionization, which concluded that the manufacturer's technology cannot clean the air at the level claimed by independent testing.

Although bipolar ionization technology has been around for decades, the lack of many rigorous peer-reviewed studies makes it difficult to assess the effectiveness of this technology in air and surface disinfection. Many of the claims of manufacturers are based on either in-house studies or external studies designed and guided by the manufacturer. A major confounding variable has been the performance of evaluations within an enclosed environment, in which the concentration of ions as well as ozone increases significantly, making it difficult to determine whether the decrease in viability of microorganisms and viruses was due to the effect of ozone or ion concentrations that are far in excess of achievable levels in a real-world setting. A study used by an electronics company to promote the effectiveness of its plasma cluster bipolar ionization technology in reducing the concentration of aerosolized SARS-CoV-2 was conducted in a 3-L enclosed chamber [16].

Table I
Log reduction of drug-resistant bacteria, *Clostridioides difficile* and fungi after 4 h of exposure to bipolar ionization

Organism	Ozone (ppm)	Ion exposure	cfu/gauze (mean of three samples)				Log reduction compared with control at 4 h	% reduction
			0	1 h	2 h	4 h		
MRSA	0.044–0.055	+		1.7×10^6	1.5×10^5	2.3×10^4	2.21	99.4
		-	3.7×10^6	4.7×10^6	3.3×10^6	3.7×10^6		
MDRP	0.055–0.066	+		2.7×10^6	2.0×10^4	$<1.0 \times 10^2$	4.76	>99.9
		-	8.7×10^6	1.3×10^7	4.0×10^6	5.7×10^6		
Clostridioides difficile	0.044	+		1.3×10^4	1.4×10^3	9.3×10^2	1.23	94.5
		-	7×10^5	5.7×10^4	2.7×10^4	1.7×10^4		
KPC-producing *Klebsiella pneumoniae*	0.022–0.044	+		8.7×10^6	3.7 x10^6	2.8×10^5	1.69	98.0
		-	1.1×10^7	7.0×10^7	8.0×10^6	1.4×10^7		
Multi-drug-resistant *Acinetobacter baumannii*	0.022–0.044	+		4.3×10^6	1.0×10^6	6.3×10^5	0.63	76.7
		-	6.7×10^6	4.3×10^6	4.3×10^6	2.7×10^6		
Candida albicans	0.022	+		5.7×10^6	3.2×10^6	4.7×10^5	0.89	87.3
		-	5×10^6	6.0×10^6	3.3×10^6	3.7×10^6		
Aspergillus fumigatus	0.033–0.055	+		Not tested	1.7×10^6	2.3×10^6	0.19	32.4
		-	3.6×10^6	Not tested	2.7×10^6	3.4×10^6		

cfu, colony-forming units; MRSA, meticillin-resistant *Staphylococcus aureus*; MDRP, multi-drug-resistant *Pseudomonas aeruginosa*; KPC, *Klebsiella pneumoniae* carbapenemase.

Table II
Log_{10} 50% tissue culture infectious dose ($TCID_{50}$) reduction of human coronavirus 229E after 2 h of exposure to bipolar ionization

Human coronavirus 229E (ATCC VR-740)	Ozone (ppm)	Log_{10} $TCID_{50}$/mL		Log_{10} reduction	%$TCID_{50}$/mL reduction compared with control
		Control	After 2 h of exposure		
	0.044	2.7	1.5	1.2	94.0

The Occupational Safety and Health Administration has set official exposure limits for ozone at ≤0.1 ppm average over an 8-h period, which is somewhat higher than the US Environmental Protection Agency's (EPA) position that ozone output of indoor medical devices should be ≤0.05 ppm [17]. In this study, ozone levels were well below 0.1 ppm and, with the exception of one reading of 0.066 ppm, ozone levels were within EPA guidelines. Furthermore, exposure of the samples was minimized by elevating the testing platform 30 cm above the acrylic chamber floor, as ozone tends to sink rather than rise. Effective ozone concentrations for micro-organisms have been reported to be 0.23–2.29 ppm for bacteria, 3–5 ppm for moulds, 0.02–0.26 for fungi, and 0.2–4.1 ppm for viruses [18–20]. While the effect of ozone on the micro-organisms tested in this study cannot be completely ruled out, the ozone levels measured were below levels reported to inactivate micro-organisms.

The study results showed a 94.4–99.9% log cfu/gauze decrease within 4 h for *C. difficile*, MDRP, MRSA and KPC-KP. As these bacteria are important pathogens associated with HAIs and are found in the healthcare environment, bipolar ionization merits further examination as a technology to minimize transmission of infections. The relatively low inactivation of MDRA conflicts with a previous study that showed a significant decrease in acinetobacter infections as well as patient colonization in an intensive care unit during a 5.5-month period using a negative air ionization technology [4]. A longer exposure to bipolar ionization in the present study may have demonstrated higher inactivation of MDRA. The testing environment in this study does not reflect a hospital ward or room; however, it would be difficult to conduct an experiment in the general environment, which would introduce other variables such as contamination from environmental micro-organisms. The ultimate usefulness of this technology may need to be evaluated in a pre- and post-intervention study to compare HAI rates.

It has been estimated that one in 25 hospitalized patients in the USA develops an infection associated with hospital care; furthermore, additional infections are seen in other healthcare settings [21]. According to the World Health Organization, seven and 15 patients, respectively, develop at least one HAI in developed and developing countries [22]. The relative contribution of fomites and droplets or aerosals in disease transmission in the healthcare setting is not clear. Beyond currently established protocols, such as personal protective equipment, aseptic technique, hand hygiene, environmental cleanliness, etc. to minimize HAIs, bipolar ionization systems to further reduce the risk of HAIs merit assessment of effectiveness as HAIs continue to occur despite the implementation of these infection control measures.

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Author contributions
IK: preparation, review and submission of the manuscript.

IK, AK and HT: data collection and data analysis.
IK and HT: data analysis, and review and submission of the manuscript.

Conflict of interest statement
None declared.

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