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Evaluation of cold plasma inactivation efficacy against different airborne bacteria in ventilation duct flow

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ABSTRACT

A full-size, experimental, ventilation ductwork was designed and set up to measure one-pass inactivation efficacies of a cold plasma installation under various practical environmental conditions. Five types of microorganisms, which are commonly associated with nosocomial infection, including *Escherichia coli*, *Pseudomonas alcaligenes*, *Staphylococcus epidermidis*, *Micrococcus luteus* and *Serratia marcescens*, were chosen and tests were conducted at four airstream velocities, ranging from 2 to 7 m/s, and two different relative humidity (R.H.) levels. The inactivation efficacies for the first three types of bacteria varied from 20% to 70%. It is interesting to note that the inactivation efficacy increased with velocity. No detectable inactivation effect was found for *M. luteus* and *S. marcescens*. The inactivation efficacy at 90% R.H. dropped to 10% of the value measured at 55% R.H. The negative ion intensities measured near the plasma installation were correlated with the airstream velocities. When compared with data from systems using conventional fibrous filters, the pressure drop measured across the plasma unit was very small. Our experiments showed that cold plasma technology has high potential to be used as an energy-efficient method for disinfection. Limitations of application are also discussed.

1. Introduction

Airborne transmitted pathogen infection can cause various diseases of significant morbidity and mortality. In highly crowded and indoor enclosed environments such as healthcare facilities, large shopping malls, commercial buildings, and public buildings, indoor pathogens shed from human may be transmitted and dispersed through HVAC systems, and may lead to cross-infections. Recent modeling studies have further showed that there was a potential risk of infection and diseases resulting from indoor airborne pathogen transmission through HVAC systems in high-rise hospitals [18,19] and offices [27]. In addition, infections can affect work productivity and cause substantial economic impacts. To reduce the risks of infection from such transmission, engineering control strategies can be implemented. At present, common solutions include filtration or dilution. Furthermore, for high risk settings, upper room ultraviolet germicidal systems have been shown to be very effective for controlling tuberculosis infection [26,32].

The minimum efficiency reporting value (MERV) is a standard index, with ratings from 1 to 16, used to rate the effectiveness of air filters for particles in the range of 0.3 μm−10 μm in size. Filters with MERV ratings from 6 to 9, are often considered as medium-grade filters used frequently in ventilation duct systems, and are effective for removing super-micron particles but not for small bacteria and viruses less than 1 μm in size [20].

For many general premises, it is not economical to use high efficiency particulate air (HEPA) filters [27]. While HEPA filters or MERV 16 filters, which are at least 99.97% efficient in removing particles of size $\geq$ 0.3 μm, are often used for clean rooms, air infectious isolation (AII) and protective environment (PE) rooms, they are considered too costly for other settings. Consequently, their use have been limited to selected high-risk areas in healthcare facilities, while many other important areas with risks of potential transmission, such as emergency departments and waiting rooms, have been neglected [3]. In addition, minimum fresh air requirements proposed by [1] to achieve acceptable indoor air quality must be taken into consideration for the filtration approach; for example, for office environments, an airflow rate of approximately 10 l/s per

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person is suggested. Although airflow rate at values higher than the suggested level can certainly have better dilution effect on pollutant concentration, higher initial costs and running costs have to be considered.

Therefore, development and application of new technologies for effective disinfection of airborne pathogens for general use, at lower costs and with sustainability in energy resources are needed to provide cleaner and healthier environments. Active "in-duct" system is one of the few technologies for disinfection of airborne pathogens used in ventilation systems. They have been available on the market and utilized for several decades. Due to their low pressure drop characteristics, UVGI and corona-type pin ionizers are the two most popular installations. Both approaches have been reported for use in cooling coils or filters, showing very high disinfection efficacies at low airstream velocities [9,22]. We report here the use of cold plasma disinfection units, which have become available commercially for in-duct applications.

Gas plasma, the fourth state of matter, is formed when a gas is ionized. Only until recently, advancement in plasma source technology allows the generation of plasma in near ambient pressure and temperature. It is called cold plasma or atmospheric pressure plasma and is generated by applying a modulated electric field through a pair of electrodes to air molecules. It is considered as an effective and energy sustainable method for air disinfection, with fewer limitations than other traditional methods [5,6,25].

While cold plasma disinfection phenomenon has been known for decades, the exact disinfection mechanisms are still not clear. It is believed that the disinfection mechanisms of cold plasmas on airborne microorganisms are likely associated with the presence of charge particles, ions, reactive oxygen species, reactive nitrogen species, UV-C, vacuum ultraviolet (VUV), and localized, periodic and short-term heating of microorganisms, as well as synergistically combined effects of these factors [5,15]. As these reactive species disappear within a few milliseconds after the plasma unit is powered off, these systems are therefore safe for human use [23].

Previous experimental studies of cold plasma predominately focused on sterilization of medical devices placed on agar plates or well plates with plasma jets [11,12]. The achieved disinfection rates as high as over 90% within a few seconds of plasma treatment have been reported [2]. Nevertheless, few studies were conducted in ventilation ducts. To our best knowledge, there was only one study investigating cold plasma disinfection effects on respiratory viruses under tube flow conditions resembling a practical ventilation system with a "reasonable" airstream velocity of 0.9 m/s [30]. However, the duct size had not been mentioned and the velocity was low for practical applications.

For in-duct applications, one or multiple tube-like plasma generators are inserted into a ventilation duct. The advantages of such systems include high airflow handling rates, very low maintenance cost and low running cost. Hence they are suitable for use in existing buildings, requiring only minimal renovation work.

One common challenge for many in-duct devices is that the disinfection efficacies decrease with airstream velocities because the doses absorbed by microorganisms would be decreased. Though the disinfection efficacies of an in-duct UV-C system for *Aspergillus versicolor* and *Mycobacterium parafortuitum* had been reported to be 75% and 87% respectively, such systems were ineffective when the airstream velocity was increased to 5.1 m/s [14]. In addition, airstream velocity relative humidity (R.H.) level is expected to be an important parameter affecting disinfection efficacy. However, such R.H. effects have not been reported previously.

Our study aims to evaluate the disinfection effectiveness of cold plasma units on airborne pathogens commonly found in hospitals that has implications for practical applications, and identify the effects of airstream velocity and R.H. on the disinfection effectiveness. Pressure drops across the unit were also measured and compared with those across conventional filters. The disinfection performance of the plasma unit was characterized by the one-pass disinfection efficacy. This will facilitate cross comparison with the conventional filter approach.

2. Materials and methods

2.1. Selection and preparation of microorganisms

Organisms of biosafety level one of different sizes, as surrogates of those commonly found in bioaerosols, were selected [33]: gram positive cocci – *M. luteus* (~1 μm) (ATCC 4698) and *S. epidermidis* (~1 μm) (ATCC 12228), and gram negative bacilli – *E. coli* (~1 × 3 μm) (ATCC 10536), *S. marcescens* (0.5 × 3 μm) (ATCC 6911), and *P. alcaligenes* (~0.7 × 3 μm) (ATCC 14909). *S. epidermidis* and *M. luteus* as human skin colonizers were selected as surrogates of pathogenic skin colonizers such as methicillin-resistant or methicillin-sensitive *Staphylococcus aureus*, while *E. coli* and *S. marcescens* as common gram negative bacilli and *P. alcaligenes* were selected as surrogates of *Pseudomonas aeruginosa*. These are commonly found in dust and on inanimate surfaces of hospital environments or formations of biofilms, and have potential clinical relevance in the transmission of nosocomial pathogens [16].

A colony of tested bacterium was obtained from stock, inoculated onto a nutrient agar (NA, Oxoid) or Trypticase soy agar (TSA, Oxoid) plate, and then incubated at 30 °C for 24–72 h (hrs). Then a colony was inoculated into 10 ml nutrient broth (Oxoid) or Trypticase soy broth (TSB, Oxoid), and incubated in an orbital shaker at 30 °C for 24–48 h to reach stationary phase. The suspension was centrifuged at 2080 × g for 30 min (mins), washed and resuspended in 10 ml of sterilized distilled water. As the airstream velocity would affect airborne bacteria concentration, in order to keep the concentration of each tested bacterium at an approximately constant value for each trial, the bacterial suspension was diluted to different ratios to obtain 10^8 to 10^9 colony-forming units (CFU) in 50 ml sterilized distilled water for nebulization.

2.2. Parameter selection

The parameters were chosen with respect to commonly encountered practical situations. Four speed settings: 2.0, 3.5, 5.0 and 7.0 m/s were selected from within the range of airstream velocities commonly used in air conditioning systems. The Reynolds numbers were between 26,500 and 92,700, corresponding to turbulent flow.

The system was tested at two R.H. levels, 50–60% and 85–90% (represented as low and high R.H. respectively), to simulate the substantial variations of relative humidity conditions throughout the year in many Southeast Asian countries. The former is typical of indoor air-conditioned condition while the latter is typical of the outdoor air for summer months in those countries.

2.3. Experimental setup

A macroscopic experimental approach was adopted. A 9-m long, 200 mm × 200 mm modular galvanized steel ductwork system was designed and fabricated. The sizes and airflow characteristics were resembled to practical scenarios. Fig. 1 shows the schematic of the experimental setup. The ductwork was housed in an air-conditioned laboratory. The room temperature and the R.H. were maintained at stable conditions of 23 °C ± 2 °C and 55% ± 5% respectively. The ductwork consisted of 5 major galvanized steel modules and a tempered-glass section. The length of module ranged from 800 to 1200 mm. While our modular design enhanced