Testing an innovative device against airborne Aspergillus contamination

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Abstract

Aspergillus fumigatus is a major airborne nosocomial pathogen that is responsible for severe mycosis in immunocompromised patients. We studied the efficacy of an innovative mobile air-treatment device in eliminating A. fumigatus from the air following experimental massive contamination in a high-security room. Viable mycological particles were isolated from sequential air samples in order to evaluate the device’s effectiveness in removing the fungus. The concentration of airborne conidia was reduced by 95% in 18 min. Contamination was reduced below the detection threshold in 29 min, even when the machine was at the lowest airflow setting. In contrast, during spontaneous settling with no air treatment, conidia remained airborne for more than 1 h. This indoor air contamination model provided consistent and reproducible results. Because the air purifier proved to be effective at eliminating a major contaminant, it may prove useful in preventing air-transmitted disease agents. In an experimental space mimicking a hospital room, the AirLyse air purifier, which uses a combination of germicidal ultraviolet C irradiation and titanium photocatalysis, effectively eliminated Aspergillus conidia. Such a mobile device may be useful in routine practice for lowering microbiological air contamination in the rooms of patients at risk.

Key words: Aspergillus, indoor air contamination, nosocomial infections, photocatalysis, titanium, mobile air purifier.
Introduction

The incidence of nosocomial mycosis is increasing rapidly [1]. Airborne transmission is the major route of contamination, especially to immunocompromised patients [2,3] and patients taking corticosteroids [4]. Various moulds, in particular Aspergillus fumigatus, are widespread and extremely virulent in this opportunistic context [5,6]; invasive aspergillosis is associated with high mortality rates [3]. The use of antifungal drugs is limited by high costs and side effects [7].

The relationship between the presence of Aspergillus in the air and outbreaks of nosocomial aspergillosis has been clearly demonstrated [8,9], and this has led to implementation of various air-quality controls [10]. A variety of technological processes have been used to treat air, including high-efficiency particulate air (HEPA) filtration and air pressure regulation [11]; however, all have been found to have technical limitations. Indeed, filtration no longer appears to be the best air-treatment process because it consumes energy and may result in the accumulation of pollutants that could be released into the air [12]. There are also problems with the growth of microbiological agents on the filter [13]. Consequently, new and innovative technologies are needed to improve air quality [14].

The development of the AirLyse AL-PR004 prototype was hosted in the medical school of Tours François-Rabelais University, France, in collaboration with industry. This patented device is a mobile stand-alone air purifier designed for continuous decontamination of indoor air in the presence of humans [15]. In contrast with existing filtration systems, the AirLyse air purifier does not have a retention function; rather, it destroys airborne particles in the ambient air. The purifier combines physical inactivation of organic residues and germicidal treatment using ultraviolet C (UVC) at wavelengths of 200–290 nm with destruction by titanium dioxide (TiO$_2$) photocatalysis [16]. TiO$_2$ photocatalysis is stable and energy efficient and has been proven to completely mineralize organic pollutants present in air and water [17] into nontoxic substances, primarily, mineral acids, carbon dioxide, and water [18]. The photocatalytic degradation process involves the production of very reactive but short-lived hydroxyl oxidants; the reactive oxygen species that are generated are directly reduced. Unlike the activated carbon, the titanium oxide is not consumed by the photocatalytic reaction, therefore allowing a continuous functioning [19]. This type of photocatalysis has been shown to effectively destroy a wide range of gram-negative and gram-positive bacteria, filamentous and unicellular fungi, algae, protozoa, mammalian viruses, and bacteriophages [17].

Among the various devices that are based on photocatalytic technology, the AirLyse air purifier is quite innovative, particularly in terms of its three-dimensional conformation, which makes it possible to treat large volumes of air. It is also convenient to use because of its mobile “plug-and-play” system, which was specifically developed for use in diverse healthcare facilities.

Here, we report on the development of an experimental model of Aspergillus environmental contamination. We then assess the AirLyse air purifier’s ability to remove A. fumigatus by measuring airborne conidia concentrations and comparing the results with those of spontaneous settling over time. This study was authorized by INRA (Institut National de la Recherche Agronomique) and La Région Centre and was performed in accordance with the principles of the Helsinki declaration.

Materials and methods

Experimental model of Aspergillus contamination

Decontamination tests were performed in a 60 m$^3$ (5 × 4 × 3 m) high-security (safety level L3) room at the Institut National de la Recherche Agronomique, Nouzilly, France (Fig. 1). The room’s heating, ventilation, and air-conditioning (HVAC) system allowed input and output air to be fully HEPA-filtered before and after the experiments. However, HEPA filtration was completely stopped during tests to avoid any airflow exchange with the outside. Between experiments, the inside air was totally renewed through the HVAC system (a process known as washout). Throughout testing, the room’s temperature and hygrometry were verified. Operators inside the experimental room wore sterile protective clothing and respirators to provide complete protection against exposure to microbiological agents.

At T0 (the time of nebulization), a dose of 10$^7$ A. fumigatus conidia was aerosolized in a single step through a central commercial jet nebulizer (Atomisor$^+$/NL9M, coupled to an Abox$^+$ compressor; La Diffusion Technique Française, Saint-Etienne, France) placed 1.6 m above the floor at the center of the room [20]. Four mobile standing fans were distributed in a circle with a radius of 140 cm around the nebulizer. The fans were started concomitantly with the Aspergillus nebulization and ran for 4 min to distribute airborne particles throughout the room.

Inoculum preparation

The A. fumigatus strain used for the experimental contamination was isolated from a patient with proven aspergillosis...
and hospitalized in the oncology–hematology department of Tours University Hospital (France). The strain is registered as no. BRFM 1827 (Banque de Ressources Fongiques de Marseille) in the World Federation for Culture Collections - Microbiological Resources Centres collection of the World Data Centre for Microorganisms (CIRM-UMR 1163 INRA, Marseille, France). The strain was plated on Sabouraud agar (Sabouraud GM+C; Becton Dickinson, Le-Pont-de-Claix, France) and incubated at 37°C for 5 days. Conidia were harvested by flooding the *Aspergillus* plate with 15 ml of a sterile solution of 0.05% Triton X100 (ICN Biomedicals, Irvine, CA, USA) in 0.9% phosphate-buffered saline (PBS; bioMérieux, Craponne, France). The suspension was collected by aspiration and centrifuged at 1700 g for 10 min. The supernatant was discarded and the pellet recovered in 50 ml PBS, and the centrifugation cycle was repeated (1700 g for 10 min). The conidia concentration was assessed in a Malassez counting chamber (Kova Slide 10; Glasstic, Garden Grove, CA, USA) and adjusted at 10^7 conidia/ml by dilution in PBS.

Assessment of different operating modes of the AirLyse air purifier

The AirLyse AL-PR004 prototype was tested in the experimental conditions described above. According to American National Standards Institute/Association of Home Appliance Manufacturers document AC-1-2002 [21], independent trials were run at the following airflows: 0 m³/h (machine turned off to allow spontaneous settling), 500 m³/h, 600 m³/h, and 1000 m³/h (machine turned on throughout each trial period).

Air sampling

Mycological samples were sequentially collected from air during every experimental procedure in order to establish the kinetics of changes in the airborne conidia concentration. Four bio-impactors (MK2 Sampl’AIR; AES Chemunex, Cranbury, NJ, USA) were placed 1.4 m away from the source of *Aspergillus* conidia and 1.2 m above the
floor (Fig. 1). The aspirated air was projected by each bio-impactor toward a malt extract agar plate (25 g malt extract, 20 g agar, 1 l water) through a grid a few millimeters above the plate. At the start of each sampling period, the bio-impactors were simultaneously turned on with a remote control; 200 l of air was sample for 2 min [22]. Baseline samples were collected at T0, when the air purifier was off; samples were then collected for 2 min at T5, T10, T15, T20, T30, T45, and T60. One hour after nebulization (T60), the fans were restarted for 5 min to redistribute any conidia that had settled on surfaces and the air was sampled at T75. A Solair 5350 portable particle counter (Lighthouse, Fremont, CA, USA) was used for instantaneous and continuous counting of total airborne particles during the experiment. The read channel that measured particles larger than 1 µm was selected in view of the size of A. fumigatus conidia (2–3 µm).

Air-quality analysis

The malt extract agar plates were analyzed at the Parasitology–Mycology–Tropical Medicine Department, University Hospital of Tours (France). After 48 h of incubation at 37 °C, the number of A. fumigatus conidia was determined for each air sample and expressed as colony-forming units (CFU)/200 l of air. Because of the number of holes in the grid placed above the plates during collection, the maximum count for any one Petri dish was n = 258 CFU/200 l. However, a count of 1 CFU may be the result of the entry of one or more conidia through the same hole in the grid; therefore, the total number of CFUs was adjusted according to a nomogram supplied by the manufacturer (Chemunex) in order to provide a more reliable count. This involved use of an algorithmic graphical calculating device to account for the statistical probability of at least two conidia having been collected at one position. By fixing the values of CFU variables according to Chemunex’s instructions, the relationship between the unfixed variables could thus be studied; a predefined table was used to obtain the corrected value N (estimated number of viable conidia impacting the plate) corresponding to the observed value n (number of CFU observed experimentally). Whenever measurements were concordant, the mean N was calculated for the four malt extract agar plates used concomitantly.

Statistical analysis

The intraclass correlation coefficient was calculated to assess concordance between the four bio-impactors using the respective N values at each sampling time. The exponential decays of the mean values of N were modeled for each rate of airflow using the following differential equation: \(N(T) = N_0e^{-\lambda T}\), where coefficient \(\lambda\) is a positive number, named the decay constant. The \(\lambda\) coefficient for each airflow rate was then compared with each other \(\lambda\) and with that for the control condition (spontaneous settling with no air treatment) using Student \(t\) test.

Results

A total of 36 sequential air samples, corresponding to four replicates (the four bio-impactors) at each of nine time points, were analyzed for the following four conditions: negative reference condition at 0 m³/h (AirLyse air purifier off) and active air treatment at 500 m³/h, 600 m³/h, and 1000 m³/h (AirLyse on). The four bio-impactors gave very similar results. The intraclass correlation coefficients were as follows: 0.987 (0.967–0.996) at 0 m³/h, 0.998 (0.996–1.00) at 500 m³/h, 0.998 (0.996–1.00) at 600 m³/h, and 0.994 (0.984–0.998) at 1000 m³/h.

Immediately after nebulization at T0, all agar plates were totally invaded by fungal colonies (\(n > 258\) colonies), indicating a high density of Aspergillus conidia in the air. This was considered to be the maximum airborne contamination (100%). When the AirLyse air purifier was off, the Aspergillus conidia density in the air decreased linearly with time: 57% at 15 min, 42% at 30 min, and still detectable 1 h after aerosolization (22.8% of initial inocula; Fig. 2). When the AirLyse system was operating at 500 m³/h, the conidia...
density in the air decreased by $1 \log_{10}$ in 7 min, $2 \log_{10}$ in 14 min, and $5 \log_{10}$ in 34 min. Elimination of 95% of conidia in the air was obtained in 18 min at 500 m$^3$/h and 600 m$^3$/h and in $<16$ min at 1000 m$^3$/h. At all airflow rates tested, 99.99% of conidia were eliminated in $<30$ min. After restarting the fans at the end of each experiment, there was no substantial increase in conidia detection (from 0 CFU/200 l to 0 after activation of the fans for both 500 m$^3$/h and 600 m$^3$/h, and from 0 to 4 for 1000 m$^3$/h; Fig. 2).

There was no significant difference between the $\lambda$ values for the different rates of airflow through the AirLyse purifier ($P > 0.05$), indicating that the decontamination kinetics did not differ according to airflow speed. Conversely, $\lambda$ values differed significantly between the negative reference condition and the three airflow treatments ($P < 0.001$).

The Solair 5350 portable particle counter was used to enumerate airborne particles smaller than 1 $\mu$m. The counts decreased by 80.6%, 88.6%, and 88.6% when the AirLyse purifier was on at 500, 600, and 1000 m$^3$/h, respectively, whereas the decrease was 45% when the unit was off. At the end of the experiment, the airborne particle count was lowest for the airflow treatment set at 1000 m$^3$/h: 357,738 counted particles $>1$ $\mu$m vs. 812,590 when the air purifier was off.

**Discussion**

*Aspergillus fumigatus* is a highly resistant fungus that contaminates air and can cause lethal infection in immunocompromised individuals. *Aspergillus* spp. represents 1%–7% of the total amount of fungi in the environment. Thus, airborne concentrations ranging from 1 to 100 conidia/m$^3$ are common both indoors and outdoors, and concentrations can reach $10^9$ conidia/m$^3$ in specific environments such as construction sites or work zones [23]. Consequently, everyone inhales conidia daily, and eradication of *A. fumigatus* from the immediate environment of susceptible patients would be extremely beneficial [24]. Indeed, the control of airborne microorganisms is a major issue in hospitals and other healthcare facilities, especially during periods of construction or demolition [25].

Although some air-cleaning systems, such as HEPA filters [11], decrease levels of microbiological contamination, several studies have revealed that a wide range of miscellaneous bacteria, including species potentially involved in human infections, may be retained on filters and subsequently released back into the filtered air [13]. Recently, new air-treatment devices have been developed to address this issue. Some of these devices incorporate three processes, each of which is independently lethal to microorganisms [26]. Airborne particles are first crashed onto a rigid three-dimensional nonocclusive self-cleaning medium, generating a physical stress [16]; they are simultaneously exposed to germicidal UVC irradiation with a reactor, allowing for a prolonged exposure; and finally, the contaminants are exposed to heterogeneous photocatalysis. The combination of these three treatments allows effective air purification [27].

We assessed the performance of an innovative mobile air purifier that is based on photocatalysis in conditions of massive air contamination by *A. fumigatus*. We developed an experimental model of fungal contamination in which aerosolization, homogenization, and sampling were standardized. The experimental room was 60 m$^3$, similar to that of a standard hospital room. The walls were smooth, which minimized particle retention and facilitated cleaning. The air was sampled at a height of 1.2 m above the floor, corresponding to the average height at which air is generally inhaled by patients who are in bed, sitting, or standing. The mycological count results were entirely consistent between the four sites in the room that were tested at every sampling time. Indeed, the intraclass correlation coefficients were, in all instances, $>0.987$ (where 1.0 indicates identical results), demonstrating the reliability of the sampling technique.

Conidia are more resistant than vegetative forms, largely because of their thick cell walls [28]. Nevertheless, we showed that they were effectively eliminated by the AirLyse air purifier. In spite of the extreme conditions of a saturated atmosphere and the massive dose of *A. fumigatus*, the device rapidly and completely purged the contaminant from the air. The mycological findings for sequential air samples were confirmed by the instantaneous measurements with the particle counter, although this enumerated all airborne particles whether or not fungal. The airflow rate through the air purifier did not have a significant effect on its efficacy, which rapidly eliminated contamination even at the minimum operational setting (500 m$^3$/h). Furthermore, at low air speed, it generated less turbulence and was quieter; this finding is of importance for the comfort of patients and healthcare workers. Also, because the photocatalytic reaction occurs at ambient temperature, the device does not lead to overheating. Using doped fused quartz, the lamps that are fitted to the AirLyse air purifier transmit up to 90% of their energy at a wavelength of 254 nm, thus avoiding ozone generation [29]. Moreover, the machine does not show chemical potential toxicity because there have been no release of volatile organic compounds (LSCE-CEA Laboratory, data not shown).

One limitation of our experimental model is that the room was totally sealed throughout the experiment. This is not the case with hospital rooms, where doors are opened frequently. To address this limitation, we reactivated the fans at the end of each experiment; this may have resuspended conidia that had settled on the floor, thus mimicking door opening and other movements. After reactivation
of the fans, the measured airborne contamination did not increase, which is consistent with the apparent total destruction of the contaminant. Also, one should note that the Tween-wetted conidia used in our experimental design could differ in their physical properties somewhat from natural aerosolization.

Aspergillus fumigatus is not the sole microorganism responsible for air-transmitted diseases. It would be useful to conduct studies with other fungi (including Scedosporium, Fusarium, and Pneumocystis), bacteria (particularly Mycobacterium tuberculosis complex, pneumococci, and multidrug-resistant bacteria), and respiratory viruses, which are emerging as the major challenge of the decade. The AirLyse air purifier combines three nonspecific germicidal processes and may be effective against all types of contaminants [26]; our experimental model may also be appropriate. Nevertheless, various conditions need to be considered based on the pathogen being targeted. For instance, it is difficult to produce aerosols of Staphylococcus aureus because its colonies are usually in biofilm aggregates. Also, it would be difficult to assess the effectiveness against contamination by influenza virus, as this pathogen does not grow directly on plates or in culture medium.

To conclude, our assay in an experimental space that mimicked a hospital room showed that, even in challenging conditions, the AirLyse air purifier was able to decrease the fungal load in the atmosphere substantially. The photocatalysis process used is relatively inexpensive and the system is self-cleaning. It may be used for diverse substrates, allowing continuous reuse; for instance, activated carbon may be used, allowing for high photodegradation rates because of its high adsorption capacity [16]. Thus, in addition to other approaches (eg, overpressure, HEPA, or outdoor air ventilation rates of 6–12 volumes per hour), the mobile AirLyse air purifier may provide additional cleaning in hospital environments, substantially and rapidly reducing indoor pathogen concentrations. Such mobile devices may therefore be an additional and convenient solution for protected areas where susceptible patients are located and for patients at moderate risk in conventional rooms during hospital renovation.

Acknowledgments

The authors thank Cécile Eclache, Cerise Kalogridis, and Roland Sarda-Esteve for technical help and Dr Eric Bailly for his involvement in the mycological analysis. The authors are also grateful to the Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, the Région Centre, and the Région Ile-de-France for financial support for the development of the experimental aerosolization chamber model. A professional scientific editing and translation company (Alex Edelman & Associates) was involved in translation of this manuscript from French to English.

Declaration of interest

M.-C. B. is chief executive officer of AirLyse. She is a medical doctor and inventor of the combined procedure of physical inactivation on a rigid support combined with UVC germicidal treatment, and final destruction of residues by photocatalysis. All other authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References


