Collecte biologique avancée de l'air pour la détection de menaces infectieuses aéroportées (BIOCAPT)

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Objectifs / Situation du sujet

The ever present threats posed by the airborne transmition of pandemic diseases (SARS, avian flu H5N1 etc…), bioterrorism (anthrax, smallpox) and nosocomial infections (Aspergillus sp.) requires new and effective strategies to capture and concentrate these species from large volumes of air. Early detection and warning will play a significant role in minimizing the consequences of such airborne threats. In certain cases, today it is possible to detect and identify biological agents in time to treat victims before the onset of fatal symptoms. In the future, increased emphasis will be placed on the ability to "detect-to-warn" (the detection of an agent cloud in time to alter air movement within a building; the ability to treat the air before it reaches the occupants; or the ability of personnel to protect themselves from exposure with physical barriers to the hazards).

In any bio-aerosol detection system an aerosol sampler is the first stage. The basic components of an aerosol sampling system are an inlet, a size fractionation device that strips unwanted larger-sized particles and debris from the distribution, and a concentrator that confines the particles on a surface or in a liquid. The output of the sampling system varies (e.g., wet or dry, concentrated or not) depending upon the requirements of the detector.

Aerosol collectors are required for all point biodetection systems and future chemical point detectors. Given that dangerous or lethal doses in aerosol form can be found at low concentration, efficient aerosol collectors must strip a large volume of air (several cubic meters) of its particulates and transfer them to a small liquid volume (a few ml) for analysis. To be effective the capture efficiency of the respirable range of particles should exceed 90 % recovery from the sampled air. Cyclones and inertial separation devices are the current state-of-the-art for airborne aerosol collection. However these processes are intrinsically very inefficient at capturing small particles and suffer from high power consumption and lack of specificity. Attempts have been made to use wetted wall cyclone aerosol collectors to increase collection efficiencies, but their power requirements are high (400-500 Watts) – driven by the need to collect particles as small as 1 micrometer.

Materials et Methods

Our recent work concerning the biological decontamination of air using non-thermal plasma and amplified electric fields has shown that electrostatic precipitation can be used for removing small particles from gas. We have also shown that a wide spectrum biological organisms (viruses, bacteria and fungal spores) ranging in size from only a few nanometers (MS2 bacteraphage) to several microns (Vaccinia) can be captured in either viable or non-viable states using electrostatic forces. The challenge is to devise a system that automatically transfers the precipitated particles into a small volume of collection fluid.

The approach used in this work consists of combining the air sampling principals of a conventional air scrubbing device with electrostatic precipitation, followed by a micro-fluidic concentrator element based on dielectrophoresis techniques. The first stage of the collector is

to incorporate a virtual impactor to segregate large particles from the flow stream. This large particle fraction can be kept for analysis or discarded. In a second stage, the contaminated air is sampled counter-current to a charged liquid electro-spray which intercepts particles, vapors, and gases, and delivers these to a liquid collection well (see Figure C.3.1)

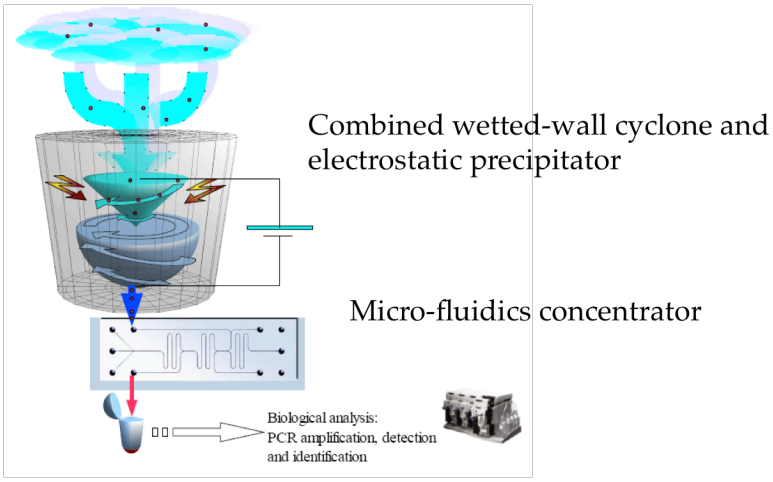
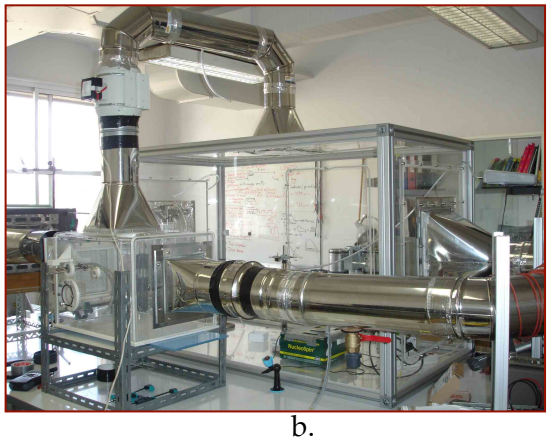


Figure C.3.1 Schematic of the technical approach used to develop a new bio-aerosol sampler.

Principaux Résultats Scientifiques

In order to test and validate samplers for the collection of active biological aerosols both innovative test equipment and protocols have been developed in this work. In particular the construction and validation of an experimental test room located at Saint Louis Hospital in Paris, France, has been completed by the ENS de Lyon team in collaboration with the laboratoire de Parasitologie-mycologie at Saint Louis Hospital, headed by Prof. F. Derioun, Chef de Service du laboratoire de Parasitologie-mycologie (1). The test room (which over 40 m2) is designed to provide a controlled environment with pressure, temperature, and humidity control, observation windows, and a controlled access SAS, Figure C.4.1 (a). A state-of-the-art biological decontamination system is used to treat the room air and thus allow us to safely produce reproducible biological aerosols in the room. This facility allows for testing biological aerosol samplers under realistic reproducible conditions. Moreover, the large size of the room permits us to simultaneously test different devices for head-to-head comparison of their performance (2-4). In parallel, small-scale laboratory testing equipment has also been developed to provide more rapid testing of individual devices and components, Figure C.4.1 (b). Testing in these controlled environments has allowed us to formulate new protocols for evaluating bio-aerosol samplers which are now being incorporated into the up and coming revised international ISO standard 14698 “Cleanrooms and associated controlled environments – Biocontamination control – General principles and methods” (2). In addition a novel method that uses the test protocol based on the Clean Air Delivery Rate (CADR) method for room air decontamination from recycle air cleaners has been developed and is being adapted by industrial manufacturers, Bertin Technologies, and the Direction General de l’Arment (DGA), Centre d’Etudes du Bouchet (6-8).

a.



b.

Figure C.4.1 (a) Image of the testing room constructed at St. Louis Hopital with a series of airborne biological samplers being tested simultaneously under controlled environmental conditions. (b) Image of the laboratory-scale testing chamber used for the evaluation of individual devices and components.

In addition to the development of testing equipment, facilities and techniques we have also designed built and tested a prototype device based on the scientific principles outlined in section C.3 and portrayed in Figure C.3.1. Photographic images of the prototype device are provided in Figure C.4.2.

The results obtained within the project have lead to both new understandings concerning the capture of micro-organisms and new tools and equipment for testing and measuring airborne levels of biological contaminates. The testing facilities and devices listed above have provided the group at ENS de Lyon with novel capabilities to work with biological aerosols in a controlled manner. In addition to using this equipment for testing and evaluation of airborne biological samplers, these tools are opening up research projects concerned with the deposition and re-entrainment of biological aerosols in an effort to understand the relationship and infection risks from air and surface sources (Thesis work supported by EDF). Furthermore, the testing protocols developed during the project are serving to guide the revision of the International ISO standard 14698 Cleanrooms and associated controlled environments – Biocontamination control – General principles and methods". Similarly, a new testing methodology based on the concept used by air cleaner evaluations, Clean Air Deilivery Rate (CADR), is being adopted by industry manufacturers of biological samplers (Bertin Technologies) and end users such as the Direction General de l'Arment (DGA), Centre d'Etudes du Bouchet.

The Flowtest™ and associated software package CosDesigner™, which form an automated controller for microfluidic systems, has been commercialized and is finding applications in industrial labs that require extremely high precision for fluid transfer.

In addition to the direct exploitation of the equipment and facilities developed, the fundamental knowledge acquired concerning how airborne micro-organisms are effected by electrostatic fields and charges will provide the template for not only designing new samplers but also decontamination technologies based on electrostatic treatment. That is, not only can the electrostatic systems improve capture efficiency, but when used under certain conditions they can also destroy the micro-organisms. The project has allowed us to map out the parameter space that will allow for capture only, without causing harm to the organisms, or capture and kill. The former is important for evaluation of the viable levels of airborne contaminates while the later can be used for their elimination.

Principaux Publications Obtenues

1. Beauchene C, Laudinet N, Choukri F, Rousset JL, Benhamadouche S, Larbre J, Chaouat M, Benbunan M, Mimoun M, Lajonchere JP, Bergeron V, Derouin F. , "Accumulation and transport of microbial-size particles in a pressure protected model burn unit: CFD", BMC InFect Dis. 2011 Mar 3;11(1):58. (Epub ahead of print).

2 . S. Barral, F. Choukri, V. Bergeron, V. Bex-Capelle, A. Nieguitsila, J. Guilot, F. Squinazi, F. Derouin "Etdue de L'Efficacite e differents Biocollecteurs après nebulisation d'Aspergillus Niger dans une Salle a Ambiance Controlee", MicrobAero2009, Oct 6-8, 2009 Narbonne, France.

3. F. Choukri, J. Menotti, C. Sarfati, El.M. Aliouat, V. Bergeron, E. Dei-Cas, A. Totet, F. Derouin, "Contamination Aerienne par Pneumocystis: Mis en Evidence et Quantification", MicrobAero2009, Oct 6-8, 2009 Narbonne, France.

4. F. Choukri, V. Bergeron, A. Totei, E. M. Aliquat, V. Bex-Capelle, S. Barral, J. Menotti, F. Derouin, "Aerobiocontamination Fongique : Exposition et Risque" 25eme Congres Français sur les Aerosols CFA 2010, 13-14 Janvier 2010, Paris France.

Conclusions

The project BIOCAPT started from the concept of combining cyclone air flows with electrostatic fields to enhance the capture efficiency of airborne biological contaminates. The effort has lead to the development of unique tools that allow for the controlled generation and handling of biological aerosols for testing purposes, along with protocols that are now being adapted by the international ISO standard commission for testing bio-samplers. A prototype sampler was developed and successfully demonstrated. Performance testing of the device lead to new knowledge of how electrical fields and charges interact with collected micro-organisms. As a result of the information and tools developed in the BIOCAPT project, two new technology development projects have emerged to create biological samplers for two specific field applications. Finally, a device and associated software for micro-fluidic control have been commercially launched and is finding use in a variety of different laboratory applications.

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4. F. Choukri, V. Bergeron, A. Totei, E. M. Aliquat, V. Bex-Capelle, S. Barral, J. Menotti, F. Derouin, "Aerobiocontamination Fongique : Exposition et Risque" 25eme Congres Français sur les Aerosols CFA 2010, 13-14 Janvier 2010, Paris France.

5. ISO 14698 standard: "Cleanrooms and associated controlled environments – Biocontamination control – General principles and methods" 2003.

6. Shaughnessy, R.J., and Sextro, R.G., "What is an Effective Portable Air Cleaning Device ? A Review", *J. Occupational and Environ. Hygiene*, Vol.3, pp. 169-181, 2006.

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