DESIGN AND EVALUATION OF A NOVEL PASSIVE BIOAEROSOL SAMPLER

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ABSTRACT OF THE DISSERTATION

Design and Evaluation of a Novel Passive Bioaerosol Sampler

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Bioaerosols are airborne particulate matter of biological origin, such as microorganisms and pollen, and any particulates shed or produced by living organisms, like pet dander and mycotoxins. A wide spectrum of adverse environmental health effects can result from exposure to these particles, such as infectious or allergic respiratory diseases. To understand and mitigate the effects of bioaerosol exposures, bioaerosol sampling must be representative of the spatiotemporal scales over which the exposures occur. However, bioaerosol sampling is typically conducted with air pumps (i.e., actively) – this negatively affects the quality of the sample and limits when and where sampling can be performed.

Passive sampling, on the other hand, does not need air pumps or external power, which makes it portable, cost-effective, and practical for conducting long-term sampling in any location. My dissertation aims to design, develop and evaluate a new passive bioaerosol sampler utilizing polarized, ferroelectric polymer films to enhance electrostatic collection of biological particles while streamlining sampling to analysis procedures.

Specifically, I aim to: 1) conceptually design the passive sampler using parallel layers of a polarized, ferroelectric polymer film to optimize collection of microorganism-sized particles. 2) Determine extraction efficiencies of spiked microorganisms from the surface of the polymer film. 3) Optimize a field-deployable prototype sampler design using a compact, calm air settling chamber. Finally, 4) perform outdoor field testing of the passive sampler to evaluate its performance.

Parallel layers of uniaxially oriented, polarized, poly(vinylidene fluoride), PVDF, with 2.25 mm wide air channels was found to significantly enhance electrostatic capture of particles in size ranges of interest for bioaerosol (~0.01 to 5 μm) with varying particle surface charge. A spiral shaped prototype sampler with a 3D-printed film holder provided user-friendly sampler setup and 100% extraction efficiency of spiked microorganisms from the surface of the PVDF and the film holder material. Through outdoor field-testing, the new sampler passively collected microorganisms comparably to the active reference sampler with an equivalent sampling rate of ~2.6 L/min. and provided better preservation of microorganism culturability. Ultimately, this research presents a novel sampling tool for bioaerosol exposure assessment, a new methodological framework for bioaerosol sampler development, and new benchmarks of success for developing passive bioaerosol sampler technology.

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CHAPTER 1. INTRODUCTION

1.1 Motivation

Bioaerosols comprise all airborne particulate matter of biological origin or activity (Cox and Wathes, 1995). These aerosols can be complex mixtures of live and dead microorganisms, such as bacteria, fungi, and viruses, and various components, residues or byproducts produced or shed by organisms, such as pollens and toxins (Baron and Willeke, 1986). Environmental bioaerosols constitute a substantial fraction (about 5 to 35% on average) of all airborne particulate matter and can be found throughout the atmosphere (Bauer et al., 2008; Elbert et al., 2007; Fröhlich-Nowoisky et al., 2009; Held et al., 2008; Hock et al., 2008; Jaenicke, 2005; Kellogg and Griffin, 2006; Smith et al., 2010; Womiloju et al., 2003). For example, about 20% of all flowering plants depend on wind pollination (Ackerman, 2000) which results in aerial dispersal and subsequent exposure to aeroallergen particles. Intercontinental dust events originating from desert regions of Africa and Asia have been shown to contribute largely to global transport of bacteria (Kellogg and Griffin, 2006), and viable bacteria have even been detected in the stratosphere 20 km over the Pacific Ocean (Smith et al., 2010). Airborne microorganisms may have significant impacts on atmospheric chemistry by influencing aerosol particle water uptake, ice nucleation, and transformation of atmospheric organic compounds by metabolic activity (Amato et al., 2005; Deguillaume et al., 2008; Krumins et al., 2014; Vaïtilingom et al., 2011).

As bioaerosol particles can have great diversity in physical dimension and shape, aerodynamic diameter is commonly used to define the size of airborne biological particles. Aerodynamic diameter is the diameter of a spherical particle with the density of water (1

g/cm^3) that has the same settling velocity as the particle of interest; thus, it standardizes particles of various shapes and densities to spheres that have similar aerodynamic properties (i.e., settling velocities) (Feather and Chen, 2003; Hinds, 1982). Bioaerosol particles typically range in aerodynamic diameter from about 0.5 to 100 μm (Blachere et al., 2009; Cox and Wathes, 1995). The common size range for bacterial aerodynamic diameter varies based on the conditions of a given environment (e.g., clean versus contaminated indoor environments), but tends to be about 0.5 to 10 μm (Gorny et al., 1999; Reponen et al., 2011). Fungal spores typically have aerodynamic diameters of 2 to 10 μm (Yamamoto et al., 2014) with smaller fragments of fungal material, like glucans and mycotoxins, being smaller than 1 μm (Gorny et al., 1999; Reponen et al., 1994). Single airborne viruses, however, have physical diameters in the nanoscale size range – about 20 to 300 nm (Blachere et al., 2009; Reponen et al., 2011). Commonly, airborne biological materials, including microorganisms, are agglomerated with non-biological particulate matter with resultant particles in the 1 to 10 μm size range (Gorny et al., 1999; Reponen et al., 2011; Yamamoto et al., 2014).

Due to the ubiquitous and diverse nature of bioaerosols, repeated, chronic human exposure to these particles occurs through inhalation, dermatologic contact, and ingestion of settled particles (Macher, 1999). As bioaerosol particles represent a broad size range of aerodynamic diameters, inhalation can result in particle deposition throughout the respiratory system, from the upper airways (nasopharyngeal) for coarse fraction particles to the lower airways (tracheobronchial/alveolar) for fine and ultrafine particles (Hinds, 1982).

Therefore, bioaerosol exposure can result in a wide spectrum of adverse health effects following inhalation, including inflammatory diseases of the airways, like asthma, infectious disease, like influenza, and multiple types of cancers from mold toxins (Douwes et al., 2003). Infectious diseases can be spread by respiratory secretions from both animals and humans (Cutler et al., 2010; Weber and Stilianakis, 2008). Legionnaire's disease has been a recurring, and recent concern due to the airborne dispersal of water droplets containing the *Legionella pneumophila* bacterium from mechanical equipment, like cooling towers and industrial air scrubbers (Mueller, 2015; Nhu Nguyen et al., 2006; Nygård et al., 2008). Bacterial aerosols are particularly a concern in the case of the transmission of antimicrobial-resistant species, like resistant *Staphylococcus* species (Messi et al., 2015) and multi-drug resistant strains of *Mycobacterium tuberculosis* (Sotgiu and Migliori, 2015).

Bioterrorism is also a potential threat for widespread dissemination of airborne pathogens (Henderson, 1999); the use of biological weapons has been called "the most important, under-addressed threat" related to terrorism (United Nations, 2006). Whether by intentional or incidental means, airborne pathogens are a threat to not just health and environment, but also prosperity and the global economy in the case of crop and livestock losses (White House National Security Council, 2010). Crop pathogens can travel around the globe by aerial dispersal and reestablish ecological epidemics in areas where host plants are seasonally absent (Brown and Hovmøller, 2002). The environmental health impacts of bioaerosol particles will become increasingly challenging to address in the future due to climate change. Global climate change will continue to impact bioaerosol exposures by impacting patterns of aeroallergen production, evolution of airborne pathogens and

potential for bioaerosol exposures through changes in land use (Boxall et al., 2009; Chakraborty and Newton, 2011; Cutler et al., 2010; Hamaoui-Laguel et al., 2015; Sadyś et al., 2015; Shea et al., 2008).

There are a number of interrelated issues in the field of bioaerosol sampling which directly result from the diversity of bioaerosol particles and exposures: 1) limited understanding of biological particle exposure-response relationships; 2) a lack of established occupational and health-related exposure limits; 3) major shortcomings of currently available bioaerosol sampling technologies; and, 4) a lack of standardization for bioaerosol sampling and analysis methods (Behzad et al., 2015; Castillo et al., 2012; Douwes et al., 2003; Duquenne et al., 2013; Eduard et al., 2012; Peccia et al., 2008). Currently, there are no bioaerosol sampling technologies available which can fully characterize all aspects of biological particles, which include factors like culturability, viability, aerodynamic diameter, and microorganism type (Reponen et al., 2011). Therefore, different types of bioaerosol samplers exist and these are tailored to specific sampling purposes and applications. However, considering the current state of bioaerosol sampling technology, and given the significance of bioaerosol environmental health impacts, bioaerosol sampling technology is a relatively underdeveloped field (Castillo et al., 2012; Eduard et al., 2012; Peccia et al., 2008; Prussin et al., 2014; Xu et al., 2011).

1.2 Background: Active versus Passive Bioaerosol Sampling

The most commonly used methods for bioaerosol sampling are active – i.e., particle laden air is pulled through the sampler using pumps to deposit particles into or onto a sampling media (Haig et al., 2016). Examples of active bioaerosol samplers include agar impactors, liquid impingers, electrostatic precipitators and filter samplers (Han and

Mainelis, 2008; Reponen et al., 2011). Depending on a particular sampler, these devices can be very effective for collection of bioaerosol samples over short durations, in environments with low biological particle concentrations, and where there is accessibility to external power (Adhikari et al., 2004; Agranovski et al., 2005; Duchaine et al., 2001; Haig et al., 2016; Jensen and Schafer, 2003; Li, 1999a, b; Thorne et al., 1992).

In contrast, passive sampling relies on collection mechanisms other than sampling pumps, like wind movement in combination with particle diffusion and gravitational deposition (Wagner and Leith, 2001b). Since no air pumps or external power are required, passive samplers can be deployed for long term durations (e.g., days to weeks), and can be used to study pollutant spatial patterns by arrangement of a widely distributed sampler network (Grosjean et al., 1995). These samplers tend to be low cost, compact, and portable which makes them ideal for assessing pollutant trends and for use as survey tools; typical sampling timeframes for using passive samplers generally range from about 8 hours (one work shift) to one month (USEPA, 2016). Due to lower maintenance and equipment costs, and no need for external power, passive sampling is more practical and less expensive than active sampling (Nothstein et al. 2000). Active sampling may be necessary for certain applications, like short term sampling where quick turnaround time is desired. However, passive sampling may be complementary to active sampling by fulfilling niche applications where active sampling is not practical or feasible, like assessment of long term bioaerosol trends across a large agricultural landscape.

1.3 Background: Post-Sampling Analyses

After collection of bioaerosol particles, the sample must be prepared and analyzed to determine sample quantity and/or identity, and the sample preparation procedures are

contingent upon the desired analysis methods. One of the most commonly used methods to analyze bioaerosol samples are culture-based methods; for example, these may include plating samples onto growth media to quantitate the number of colony forming units as indication of sample culturability (Lin et al., 1999b) or by directly sampling onto growth media, such as with agar impactors (Chang et al., 1995).

Since a significant portion (~7 to 91%) of airborne microorganisms may not be culturable (Palmgren et al., 1986), and even non-viable bioaerosol particles may cause health effects such as allergic reactions (Douwes et al., 2003), non-culture based methods are also commonly employed. Non-culture based analysis methods include microbial analyses which assess the numbers or quantities of microbes, such as by staining and counting with microscope, or quantification of microbial components, like endotoxins (Cox and Wathes, 1995). Analysis methods can also be used to identify microbes, such as antibody-based detection of specific microbial antigens (Cox and Wathes, 1995). Non-culture based analysis methods commonly include: real-time quantitative polymerase chain reaction (qPCR) (An et al., 2006), staining and epifluorescence microscopy (Palmgren et al., 1986), staining and counting by flow cytometry (Chen and Li, 2005), endotoxin measurement (Duquenne et al., 2013), high throughput pyrosequencing (Hoisington et al., 2014), and ATP bioluminescence (Seshadri et al., 2009). Most of these commonly used methods are aqueous based. Therefore, the development of new bioaerosol sampling technology would benefit from focusing on integration of sampling activities with particle removal and analysis methods that are aqueous based. Current trends in bioaerosol analysis research include investigations into how to improve microbial analyses with low concentration samples (Luhung et al., 2015) and how to

perform analyses faster with the ultimate goal of having real-time pathogen sampling and detection (Usachev et al., 2015).

1.4 Background: Limitations of Active Bioaerosol Sampling and the Need for Passive Sampler Development

There are several major drawbacks to the use of active sampling technology. First, active sampling results in structural and metabolic damage to the collected microorganisms via sampling stressors, like desiccation and impaction; this decreases the ability to identify and quantify the sample (Stewart et al., 1995; Zhen et al., 2013). Second, active sampling increases logistical difficulties. Portable pumps used with personal samplers are cumbersome, provide limited flowrates, and the wearing of sampling pumps may affect exposure estimates by causing the wearers to modify their typical behaviors (Cherrie et al., 1994; Wood, 1977). Pumps used with area monitors, are big, noisy, and usually require external power (Cartwright et al., 2009; Plog, 2001). Active bioaerosol sampling, such as the use of agar impactors, requires continuous maintenance and monitoring as the sampling media can overload and desiccate (Jensen and Schafer, 2003; Li, 1999b). Thus, active bioaerosol sampling is an expensive and labor-intensive endeavor that typically requires technically trained staff (Cartwright et al., 2009).

Limitations associated with active sampling have also led to reliance on centrally located pollutant monitoring and the use of alternative exposure surrogates, like distance of home from pollutant sources. However, this leads to poor estimation of personal exposures and exposure misclassification (Baxter et al., 2010; Meng et al., 2005). The use of exposure modeling instead of personal sampling is subject to the quality and range of the input data which may not extrapolate accurately (Briggs, 2005). In order to understand

and characterize population exposures, both personal and area sampling are needed to see individual exposure differences (Chen and Hildemann, 2009; Meng et al., 2005; Toivola et al., 2002). Proper exposure assessment increases the ability to detect exposure-response relationships which can have resounding public health impacts when large numbers of people are affected (Baxter et al., 2010; Briggs, 2005). Ultimately, the drawbacks of active sampling limit when and where bioaerosol sampling can be performed. So, reliance on active sampling has contributed to the lack of understanding about how bioaerosols impact health and environment (Douwes et al., 2003; Kuske, 2006; Macher, 1999; Xu et al., 2011).

1.5 Background: Passive Particle Capture Mechanisms and Limitations

There are multiple passive samplers for gaseous air pollutants (Krupa and Legge, 2000; Shoeib and Harner, 2002). However, there are currently few passive samplers specifically designed and/or tested for bioaerosols with demonstrated feasibility to conduct the full spectrum of sampling activities, including post-sampling analyses (Haig et al., 2016). The most common example is the agar settling plate which exposes growth medium (agar) to the atmosphere to collect microorganisms settling by gravity (Cox and Wathes, 1995). Other passive bioaerosol samplers utilizing the gravitational settling of particles include the Personal Aeroallergen Sampler (PAAS) (Yamamoto et al., 2006) and the electrostatic dustfall collector (EDC) or electrostatic dust cloths (Kilburg-Basnyat et al., 2015; Noss et al., 2010; Noss et al., 2008). While it is not necessarily air sampling, surface swabbing, washing, wiping and vacuum sampling are also often used as indicators of past presence of airborne particles since airborne particles with sufficient settling velocity eventually deposit onto surfaces, particularly in low turbulence environments (Favero et

al., 1968; Kirschner and Puleo, 1979; Lewandowski et al., 2010; Rose et al., 2004; Sanderson et al., 2002; Sonnergren et al., 2013; Thorne et al., 2005; Tovey et al., 2003).

There are major challenges associated with these currently available passive bioaerosol sampling methods. For example, agar settling plates will indicate culturable bioaerosol fraction only, and EDCs require a rigorous extraction protocol to remove collected particles for analyses (Adams et al., 2015). Furthermore, passive sampling methods relying on particle settling are recommended to be used for biological particles > 5 μm in diameter (Yamamoto et al., 2011). This size range, however, excludes all single viruses and bacteria, most fungi, and smaller fragments or byproducts of biological particles (Reponen et al., 2011). Regardless, due to simplicity and cost effectiveness, passive samplers, like the settling plates, have sometimes been preferred over active samplers for assessing the presence of biological particles in resource-limited situations (Andon, 2006; Khawcharoenporn et al., 2013).

Passive sampler concepts developed for general aerosol particle collection (i.e. non-biological particulate matter) (Seethapathy et al., 2008) could, in theory, be adapted for use as passive bioaerosol samplers. Passive collection mechanisms that have been used include particle deposition, such as the Wagner-Leith (UNC) Sampler and Einstein-Lioy Deposition Sampler (Einstein et al., 2012; Wagner and Leith, 2001a; Wagner and Leith, 2001b; Wagner and Leith, 2001c; Wagner and Marcher, 2003), and particle deposition with sticky foil capture, such as Vinzents Passive Sampler (Vinzents, 1996). But, the collection mechanisms of these samplers have limitations, such as inability to collect enough particles within single work shifts (8 hr sampling), exclusion of particles within size ranges of interest, and incompatible analysis procedures, like requiring extensive modeling to

estimate aerosol concentrations. These limitations make these collection mechanisms and devices too impractical and irrelevant for comprehensive bioaerosol sampling. Real-time bioaerosol sensors, such as infrared spectrometers and WIBS (Wideband Integrated Bioaerosol Sensor) (Healy et al., 2012; O'Connor et al., 2013; Robinson et al., 2013), are also being developed, but these do not fill the void for bioaerosol collection. These sensors detect the presence of biological particles; however, in realistic scenarios, this technology has not been proven capable yet of identifying microorganism species (Blecka et al., 2012; Perring et al., 2015).

1.6 Background: Passive Bioaerosol Sampling using Electrostatic Collection

Passive bioaerosol sampling by gravitational settling could be replaced or enhanced by aerosol capture via electrostatic forces, as it is done in electret-type passive aerosol samplers. Electrets have been previously designed and successfully used for passive aerosol sampling (Brown et al., 1996; Brown et al., 1995; Brown et al., 1994a, b; Burdett and Bard, 2007; Thorpe et al., 1999). Aerosol particles are in constant motion in the environment due to gravitational settling, Brownian motion, and wind; particles that come close enough to a collection substrate of opposite charge will be acted upon by the substrate's electric field and attracted to it (Hinds, 1982). While most aerosol particles eventually exhibit Boltzmann equilibrium charge distribution (Hinds, 1982), bioaerosol particles typically have more charge than non-biological particles. This is due to charge imposed by dispersion processes as well as a high natural charge associated with cellular metabolic activity (Mainelis et al., 2001). Therefore, application of passive electrostatic sampling for bioaerosol capture is particularly appealing (Mainelis et al., 2002; Mainelis et al., 2001).

Airborne microorganisms, including vegetative bacteria and bacterial and fungal spores, have been shown to have positive and negative surface charges, and most environments seem to have bioaerosol populations with a net negative charge (Lee et al., 2004). Outdoor culturable bacterial aerosol has been shown to have normally distributed charge levels with a peak at about 21-29 elementary charge units, while indoor culturable bacterial aerosol tends to be skewed with a peak at about 46-92 elementary charge units (Wei et al., 2014). Mainelis et al. (2002) showed that even without applying additional charge to aerosolized particles, *Pseudomonas fluorescens* bacteria carried a higher net negative charge (up to 1.3×10^4 +/- elementary charges per bacterium) than non-biological control particles of the same size (0.65-0.8 μm NaCl with <18 elementary charge units on average). Yao and Mainelis (2006) have shown that the innate charge carried by bacterial and fungal aerosols can be effectively utilized for their collection when using an active sampler with low sampling flow rate. Furthermore, removal of mixed pollutant particles from the air of a composting facility via electrostatic mechanisms (a dielectric barrier discharge reactor) showed that bioaerosol particles were removed with higher efficiency than non-biological particles (Park et al., 2011)).

However, direct application of electret-type materials for passive bioaerosol collection would be problematic because the injected charge on the electret material will be lost over time (Burdett and Bard, 2007). Electrets have been reported as being permanently polarized (Brown et al., 1994b; Seethapathy et al., 2008), but this is incorrect for corona poled polypropylene material like that described by Brown et al. (1994a). Corona poled polypropylene is well known for its ability to asymmetrically store (trap) electrical charge and produces an electric field at the trapping surface from these

charges. The material is not polarized and the stability of the electric field depends on how well (deeply) the charges are trapped. Over time, these trapped charges will slowly diffuse away or quickly fade through use as the charge is neutralized by captured particles (Burdett and Bard, 2007; Zhang et al., 2012). This was observed during evaluation of the electret sampler developed by Brown et al. (1994b); the sampler was shown to provide limited qualitative aerosol data because the electrets quickly lost a substantial fraction of charge when used in the field (50 to 95% across several days). Therefore, the collection efficiency continuously decreased throughout use as well (Burdett and Bard, 2007).

1.7 Background: Application of Ferroelectric Polymer Films to Passive Bioaerosol Sampling

This dissertation seeks to overcome the current limitations of passive bioaerosol sampling by investigating the application of a different class of materials - polarized ferroelectric polymer films - as the basis for a passive bioaerosol sampler with enhanced electrostatic collection. Example materials include polarized, ferroelectric, polymer films, such as films of uniaxially oriented poly(vinylidene fluoride) (PVDF) or its copolymers with trifluoroethylene. A ferroelectric film (i.e. a film whose direction of electric polarization can be switched), which has been polarized by application of an external electric field, will maintain that polarization when the field is removed, until it is heated to high temperatures approaching the film's Curie temperature (Nalwa, 1995). This is the temperature at which the polymer properties (ferroelectric crystallization) change and the polarization of the polymer is lost (Dargaville et al., 2005). Polarized, uniaxially oriented PVDF, for example, has a Curie temperature of about 167°C and will not begin to lose

polarization until heated to above 100°C (Nalwa, 1995). Thus, these films are permanently polarized for typical indoor and outdoor environmental sampling applications. Due to the films' polarization, one side produces a negative electric field at its surface and the other side a positive electric field at its surface (Nalwa, 1995; Wang et al., 1988). Ferroelectric polymer films have a stable polarized conformation even when exposed to chemical and ultraviolet radiation stressors because they are fluoro-polymers (Nalwa, 1995) . Ferroelectric polymer films and other ferroelectric materials have been optimized for various applications, such as ultrasound focusing, transducers, actuators, pressure/temperature sensors, aerospace mirrors and as the membrane for the Western Blot analytical technique (Dargaville et al., 2005; Kurien and Scofield, 2006; Qiu, 2010). This dissertation investigates the novel application of these polymer films to passive biological particle sampling.

It is theorized by this thesis that orientation of ferroelectric films as parallel layers with the positive and negative polarization sides of the films facing each other across fixed air gaps will result in the electric fields from both film surfaces pointing in the same direction - the total field in the gap would then be equal to the sum of the fields from each film. Thus, positively charged particles would be attracted by the electric field of one of the films and repelled by the electric field of the other. The negatively charged particles would be collected by the same mechanisms but would deposit on the opposite side of the films. Compared to passive sampling with electrets, the use of polarized, ferroelectric polymer films for passive bioaerosol sampling may provide more predictable, sustainable collection efficiency throughout use in the field over long-term sampling durations.

1.8 Definitions for Short Term and Long Term Bioaerosol Sampling

It is necessary to define the terms for short term and long term air sampling, as these concepts are recurrent themes of this thesis. Currently, there is no clear definition in the literature regarding what is considered to be short versus long term air sampling. The decision on sampling time depends on the chosen sampling method, its volumetric sampling rate, the sensitivity of the chosen analysis method, and the concentration levels in the sampling environment (Justus, 2011). Therefore, sampling time is dependent upon the sampler and environmental conditions as the time needed to obtain a measureable sample quantity will vary (Justus, 2011). For example, a 15 minute sample period may be too long for an agar impactor leading to desiccation of the agar media and overloading of the agar surface (Li, 1999b). However, a 15 minute sample with a passive deposition sampler would be too short to collect an adequate number of particles for analyses in most indoor and outdoor environments (Wagner and Leith, 2001a). Additionally, air sampling time for exposure assessment should be conducted at times for which exposures are of most concern; pollutant concentrations in a given location may vary based on factors like time of day and current activities at the site (Ott et al., 2007).

For this thesis, short term sampling is defined as sampling for less than 8 hours (a single work shift) and long term sampling is defined as sampling $\geq$ 8 hours. The timeframe for which passive sampling is considered to be the ideal option for assessing personal and environmental exposures is one day to two weeks, with the extremes of this range being 8 hours to one month (USEPA, 2016). This is the sampling time range for which passive samplers should be a feasible option under requisite concentration conditions (USEPA, 2016). While not explicitly defined in the literature, short term air sampling is commonly referred to as grab sampling with sampling periods of several minutes to several hours

(Justus, 2011; Lin et al., 1999a; Peach and Carr, 1986). Long term air sampling is commonly referred to as integrated or averaged sampling with sampling periods of several hours to several weeks (Brown et al., 1993; Justus, 2011; Lin et al., 1999a; Peach and Carr, 1986).

1.9 Hypothesis and Specific Aims

Hypothesis: Polarized, ferroelectric polymer films can be used to design a passive bioaerosol sampler that meets the following *final sampler goals*:

1. Collection of the full size spectrum for bioaerosol particles, ranging in size from the nano-scale, which includes single viral particles, to the micron-sized scale which includes bacteria, fungi, pollen, and larger biological/non-biological particulate matter agglomerates;
2. Statistically significantly greater extraction efficiency of collected microorganisms as compared to control materials typically used for active and passive bioaerosol sampling. For this thesis, extraction is defined as the removal of particles and microbes from the surface of a given test material by use of washing/elution procedures.
3. Statistically significantly greater passive bioaerosol particle capture as compared to gravity-based passive deposition controls; and,
4. An equivalent sampling rate calibrated against an active reference control sampler statistically significantly greater than 100 mL/min (the approximate equivalent sampling rate of the Personal Aeroallergen Sampler and the highest reported value for a previously developed passive aerosol sampler (Yamamoto et al., 2011)).

The *overall goal of this dissertation* is to design and evaluate a novel passive bioaerosol sampler that provides improved capture of bioaerosol particles utilizing the electrostatic collection method. The *main objective of this dissertation* is to test the presented hypothesis by addressing the following *specific aims*:

1. Conceptually design the passive sampler using parallel layers of a polarized, ferroelectric polymer film by determining the film type and air channel width that optimizes collection efficiency of microorganism-sized test particles (approximately 0.01 to 5 μm).
2. Determine extraction efficiency of spiked microorganisms from the surface of the polarized, ferroelectric polymer film type chosen through Specific Aim 1 versus control materials commonly used for active (PTFE filters) and passive (electrostatic dust cloths) bioaerosol sampling.
3. Compare collection efficiencies of mixed bacterial aerosol using field-deployable prototype sampler designs in a compact, calm air settling chamber.
4. Perform outdoor field testing of the optimized passive sampler to evaluate its performance in terms of equivalent sampling flow rate versus active and passive control samplers.

1.10 Qualitative Sampler Goals

A large focus of this dissertation is to design a new sampler that improves the user experience of passive bioaerosol sampling. The user experience includes user interaction with a given product, including the product's tangible value and usefulness, as well as the assessed value and desirability of using such a product (U.S. Department of Health and Human Services, 2016). Throughout the entire sampler development, particular emphasis

is placed on streamlining the full spectrum of sampling activities from sampler setup and deployment to post-sampling analyses. Focus on the end-to-end (sampling to analysis) process has been previously called to attention as a need for future development and evaluation of bioaerosol sampling technology (Dybwad et al., 2014). Therefore, this research addressed sampler design and user-friendliness as part of the overall goal for a final sampler product. Design thinking is a methodological framework for innovation that focuses on human-centered design; the end result is the development of products and practical solutions that people actually want to use (Brown, 2008).

1.11 Dissertation Overview

The research presented in this dissertation focuses on the design, development and evaluation of a novel passive bioaerosol sampler using polarized, ferroelectric polymer films. This is a novel approach to passive particle capture using electrostatic collection. The ultimate goal is to provide a new tool for bioaerosol sampling and exposure assessment.

Chapter 1 describes the motivation for this research, and the current limitations facing the bioaerosol sampling field. Electrostatic collection is introduced as a passive sampling method that may overcome collection efficiency challenges associated with other passive sampling methods.

Chapter 2 investigates the use of polarized ferroelectric polymer films as the basis for a passive bioaerosol sampler with enhanced electrostatic collection. The air channel width between parallel film layers is varied to investigate how to optimize particle collection efficiencies and the extraction efficiency of collected particles from the film surfaces is investigated as well. Overall, this chapter demonstrates that a ferroelectric

polymer film, poly(vinylidene fluoride), or PVDF, appears to be a strong candidate for developing the passive bioaerosol sampler with enhanced electrostatic capture of particles in size ranges of interest for bioaerosol with varying particle surface charge. Chapter 2 provides the conceptual framework for developing and validating a field-deployable passive bioaerosol sampler using polarized ferroelectric polymer film.

Chapter 3 presents testing of different prototype designs for the passive bioaerosol sampler concept by incorporating PVDF film into 3D-printed film holders. This chapter presents the first adaptation of a calm air settling chamber to bioaerosol sampler prototype studies with a coefficient of variation of number of settling bacteria onto a rotating platform of < 10%. The extraction efficiencies of spiked microorganisms off PVDF, 3D-printed film holder materials, and controls (PTFE filters and electrostatic dust cloths) are also investigated. Overall, this chapter provides key evidence for the proof of concept in using the prototype passive sampler to significantly enhance the capture of bioaerosol particles compared to passive deposition alone. Not only does Chapter 3 present the final refinements to the sampler's design prior to field testing, it may also serve as a framework for future prototype testing of new passive bioaerosol sampler technology.

Chapter 4 presents the outdoor field testing and evaluation of the passive bioaerosol sampler. A trade name is also presented for the sampler: the Rutgers Electrostatic Passive Sampler (REPS). Four 10 day long outdoor field test campaigns were conducted in highly varied conditions to compare collection efficiencies of REPS to active controls (Button Aerosol Samplers) and passive controls (PTFE settling filters and agar settling plates). This chapter presents results that suggest REPS passively collects microorganisms comparably to an active sampler over long sampling durations while providing better preservation of

microorganism culturability. Overall, this chapter presents the first application of REPS in highly varied meteorological conditions with broad applicability to different sampling environments and conditions.

The summary, implications, and future directions are presented in Chapter 5. The performance of the passive sampler during outdoor field testing is evaluated in reference to the final sampler goals and is compared to previously described passive aerosol samplers. Examples are provided for the different types of potential applications for REPS passive sampler technology. Practical and methodological implications which may stem from the diverse sampler applications are presented, and future directions for research are described in light of the major limitations discovered during my research.

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CHAPTER 2. DESIGNING A PASSIVE BIOAEROSOL SAMPLER CONCEPT USING POLARIZED FERROELECTRIC POLYMER FILM

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2.1 Abstract

This research investigates the use of polarized ferroelectric polymer films as the basis for a passive bioaerosol sampler with enhanced electrostatic collection. Using a parallel plate microchannel setup, collection efficiencies for charge neutralized and non-charge neutralized Arizona Road Dust particles in viral, bacterial and fungal size ranges (14 to 6000 nm) were compared for ferroelectric polymer films - poly(vinylidene fluoride) (PVDF) and a PVDF trifluoroethylene copolymer (77% PVDF, 23% TrFE) - versus non-ferroelectric film controls (polyvinylchloride and polyfluoroalkoxy alkane). Air channel widths between parallel sheets of these films were varied from approximately 1.00 mm to 6.25 mm to determine the optimum separation for collection of particles flowing through air channels with typical indoor air velocity. The extraction efficiency of collected particles off these films as a function of ultrasonication time was also investigated. The ferroelectric polymers gave 13% to 30% greater particle collection efficiencies (on absolute scale) across all tested particle size ranges (Welch's $F(5, 51.087) = 348.201$, $p < 0.001$) compared to other materials. Collection efficiencies of the ferroelectric polymers were significantly greater for both highly charged particles and particles exhibiting Boltzmann charge equilibrium. For parallel sheets of PVDF,

significantly greater collection efficiency (~30% on absolute scale) was obtained using an air channel width of 2.25 mm compared to other tested widths (Welch's $F(3, 50.453) = 40.094$, $p < 0.001$). Approximately 100% extraction efficiency of collected particles off PVDF film surface was obtainable using an 8-minute ultrasonication. Thus, poled PVDF, a ferroelectric polymer film, appears to be a strong candidate for developing a passive bioaerosol sampler with enhanced electrostatic capture of particles in size ranges of interest for bioaerosols with varying particle surface charge. This study is the conceptual framework for a larger research project aimed at developing and validating a field-deployable passive bioaerosol sampler using polarized ferroelectric polymer.

2.2 Introduction

Bioaerosols encompass airborne particulate matter of biological origin, like viable and nonviable microorganisms, pollens, their fragments, and by-products. Airborne biological particles are ubiquitous and can cause a wide range of environmental health effects (Douwes et al., 2003). Human health impacts include allergic and non-allergic respiratory diseases and infections from pathogenic or opportunistic microbes (Cole and Cook, 1998; Samadi et al., 2013). Environmental impacts include crop and livestock losses which can occur as a consequence of long-distance aerial pathogen transport (Brown and Hovmøller, 2002). These impacts may become increasingly difficult to manage in the future because of climate change (Chakraborty and Newton, 2011; Hamaoui-Laguel et al., 2015; Sadyś et al., 2015).

These broad-ranging environmental health effects call for practical, widely deployable bioaerosol monitoring and exposure assessment tools. Commonly used bioaerosol samplers, such as impactors and impingers, are based on active sampling. E.g.,

a pump and a power source are needed to provide air flow through a collection device resulting in particle deposition onto or into a sampling medium (Reponen et al., 2011). This can be problematic for several reasons. First, active sampling is associated with structural and metabolic damage to collected microorganisms, which can hinder their identification and quantification (Stewart et al., 1995; Zhen et al., 2013). Second, reliance on pumps can be logistically complicated. Stationary pumps tend to be large and noisy, and mains power is usually not easily available in the field; portable (personal) pumps can be used, but this increases size and weight of sampling equipment and limits sampling time due to battery capacity (Cartwright et al., 2009; Plog, 2001). Regardless of battery capacity, active bioaerosol samplers typically have limited sampling times due to sampler medium overload and/or desiccation (Jensen and Schafer, 2003; Li, 1999). Third, the use of active sampler technology to perform bioaerosol exposure monitoring is an expensive and labor-intensive endeavor which requires technical staff to frequently replace sampler media in multiple samplers across a given location (Cartwright et al., 2009).

In contrast to active sampling, passive sampling collects air pollutants without an air pump or external power, and it requires less personnel time and financial cost than active sampling (Nothstein et al., 2000). These features make passive samplers ideal for assessing personal and environmental exposures over long timeframes and across large spatial scales (USEPA, 2016). There are multiple passive samplers for gaseous air pollutants (Krupa and Legge, 2000; Shoeib and Harner, 2002) and some passive air samplers designed for general particulate matter (Seethapathy et al., 2008). However, there are currently few passive samplers specifically designed and/or tested for

bioaerosols with demonstrated feasibility to conduct post-sampling analyses. The most common example is the agar settling plate which exposes growth medium (agar) to the atmosphere to collect microorganisms settling by gravity (Cox and Wathes, 1995). Other passive samplers utilizing the gravitational settling of bioaerosols include the Personal Aeroallergen Sampler (PAAS) (Yamamoto et al., 2006) and the electrostatic dustfall collector (EDC) or electrostatic dust cloths (Kilburg-Basnyat et al., 2015; Noss et al., 2010; Noss et al., 2008).

There are major challenges associated with these currently available passive bioaerosol samplers. For example, agar settling plates will indicate culturable bioaerosol fraction only, and EDCs require a rigorous extraction protocol to remove collected particles for analyses (Adams et al., 2015). Furthermore, passive samplers relying on particle settling are recommended to be used for biological particles > 5 μm in diameter (Yamamoto et al., 2011). This size range, however, excludes all single viruses and bacteria, most fungi, and smaller fragments or byproducts of biological particles (Reponen et al., 2011). Regardless, due to simplicity and cost effectiveness, passive samplers, like the settling plates, have sometimes been preferred over active samplers for assessing the presence of biological particles in resource-limited situations (Andon, 2006; Khawcharoenporn et al., 2013).

Passive aerosol sampling by gravitational settling could be replaced or enhanced by aerosol capture via electrostatic forces, as it is done in electret-type passive aerosol samplers (Brown et al., 1994a). Application of passive electrostatic sampling for bioaerosol capture is especially appealing since bioaerosols may have a higher surface charge than non-biological aerosols due to both a natural microbial membrane charge and

a charge imposed by dispersion processes (Mainelis et al., 2001). Natural bioaerosol charge has been successfully used to capture bioaerosols by an electrostatic precipitator that did not charge the incoming particles (Yao and Mainelis, 2006). However, direct application of electret-type materials for passive bioaerosol collection is problematic because the injected charge on the electret material will be lost over time resulting in decreasing particle collection efficiency (Burdett and Bard, 2007). This chapter seeks to overcome the current limitations of passive bioaerosol sampling by investigating the application of a different class of materials - polarized ferroelectric polymer films - as the basis for a passive bioaerosol sampler with enhanced electrostatic collection.

2.3 Passive Bioaerosol Sampler Concept

Figure 2-1 depicts the concept for how parallel oriented layers of a ferroelectric polymer film may be used to electrostatically capture charged bioaerosol particles. The electric field lines of the sampler extend outward which increases the exposure of the sampler's electric field to airborne particles. In concept, the sampler can be made using pairs of any polarized ferroelectric films. For example, polarized, ferroelectric, polymer films such as films of uniaxially oriented poly(vinylidene fluoride) (PVDF) or its copolymers with trifluoroethylene can be used in pairs to electrostatically attract charged particles out of the air and onto the films' surfaces. Particles are attracted to and deposited onto the film's poled surface of opposite polarity to the charge on the particle. The electric field strength on the outside surface of a polarized, ferroelectric film can be estimated by the equation for the electric displacement field, D, in a dielectric material (Nalwa, 1995):

$$D = \varepsilon_o E + P$$

Where:

D = electric displacement field, C/m^2

ε_o = vacuum permittivity ≈ air permittivity = $8.85x10^{-12}$ C/V·m

E = electric field strength, V/m

P = polarization density, C/m^2

Is is assumed that D is continuous across the films interface with air, and inside the film E = 0 since there is no electric field inside the film. Thus, D = P inside the film. On the outside surface of the film, there is no polarization as the polarization is inside the film; therefore, P = 0 on the outside of the film, and $D = \varepsilon_o E$. Since D on the inside surface of the film is equal to D on the outside surface of the film, $P = \varepsilon_o E$ on the outside of the film and $E = P/\varepsilon_o$ (Nalwa, 1995). The polarization density of poled PVDF, for example, has been reported as 90 mC/m^2 (Nalwa, 1995). Therefore, the electric field strength on the outside surface of poled PVDF film is estimated to be 10^{10} V/m. Since this value is directly at the film surface, the electric field in the air channel between the parallel layers of the film will be lower. The measurement of this electric field in the air channel is beyond the scope of this thesis as there are no standard measurement protocols for electric fields between layers of thin films; development of such a measurement protocol should be addressed in future research.

When a charged particle is in an electric field, it experiences a force for which it will eventually reach a terminal (maximum) velocity of motion as opposite polarities attract and like polarities repel (Hinds, 1982; Kulkarni et al., 2011). This force, F_E, on a particle with n elementary units of charge, e, in an electric field, E, is (Equation 15.8, (Hinds, 1982)):

$F_E = neE$

Where:

n = the total number of elementary charge units on a particle

e = the elementary unit of charge, 1.602×10^{-19} C

The terminal electrostatic velocity (V_{TE}) is typically described for particles in the Stokes region where it is assumed there are low air velocities and small particles (Hinds, 1982; Kulkarni et al., 2011). So, V_{TE} is obtained by equating the electrostatic force on a particle to the drag force it experiences, F_D, which is the total resistive force on a spherical particle as it moves through a viscous fluid at a certain velocity. This equation for F_D is also known as Stokes Law (Equation 3.18, (Hinds, 1982)):

$$F_D = [3\pi\eta V d]/Cc$$

Where:

η = air viscosity, Pa·sec

V = particle velocity, m/s

d = particle diameter, μm

Cc = the Cunningham slip correction factor for small particles (particles approximately < 1 μm)

By equating $F_E = F_D$, the resultant V_{TE} is (Equation 15.15, (Hinds, 1982)):

$$V_{TE} = [neECc]/[3\pi\eta d]$$

Thus, the ease at which a particle will reach V_{TE} (i.e., its electrical mobility), is directly proportional to the strength of the electric field and the number of charges on that particle, and inversely proportional to the particle's diameter. Smaller and more highly charged particles will therefore tend to be more easily captured by a passive sampler utilizing the electrostatic capture mechanism.

Particles with little or no electrostatic charge can also be collected as they deposit due to gravity and diffusion. Larger, denser particles will tend to settle more easily than smaller particles as these particles have greater mass and therefore greater settling velocities (Ackerman, 1997; Hinds, 1982), but more turbulent environmental conditions will hinder particle settling. Particle diffusion is the primary transport mechanism for particles < 0.1 μm as these particles undergo Brownian motion (Friedlander, 2000; Hinds, 1982); Brownian motion occurs by the irregular movements of particles in still air caused by random collisions with gas molecules (Hinds, 1982). According to Fick's first law of diffusion, the diffusion of aerosol particles occurs as the net transport of particles from regions of higher to lower particle concentration (Friedlander, 2000; Hinds, 1982). If there is no concentration gradient, then there is no net mass transfer of particles as the Brownian motion would be the same in all directions (Friedlander, 2000; Hinds, 1982).

Figure 2-1 depicts these different forces and mechanisms which influence particle capture for an imaginary positively charged particle between the sampler films. Whether or not a particle deposits onto the film surface will be determined by the particle's characteristics, such as net charge and aerodynamic diameter, and environmental conditions of sampling, such as wind velocity. The investigation presented here had three main objectives: 1) Compare the particle collection efficiency of polarized ferroelectric polymers versus non-ferroelectric materials for collection of particles from 14 nm to 5 μm; 2) Investigate the particle collection efficiencies of ferroelectric polymer films as a function of air channel width between parallel sheets of such films; and 3) Investigate extraction efficiency of collected particles from the ferroelectric films for subsequent analyses.

The first objective will investigate if the electric field associated with polarized, ferroelectric films can be utilized to statistically significantly enhance capture of particles simulating typical particle sizes and charge levels in the ambient environment. If poled, ferroelectric polymer films do enhance electrostatic particle capture, the second objective investigates how to optimize the electric field strength between parallel film layers for particle capture. Because there is a wide distribution of bioaerosol particle sizes in the ambient environment, it is important for this phase of experimentation to investigate optimization of particle capture using test particle sizes that represent the full size spectrum for bioaerosol particles. Hence, the first and second objectives of this chapter address the first goal for the final sampler. The third objective addresses the second final sampler goal which is to have greater extraction efficiency of collected microorganisms as compared to control materials. This is important for the final sampler so that analysis procedures can be streamlined and simplified.

2.4 Materials and Methods

2.4.1 Experimental Setup

Figure 2-2 illustrates the experimental setup used to test the particle collection efficiencies of the ferroelectric films and controls for the initial developmental stages of the passive bioaerosol sampler. This wind tunnel setup was used because the materials' particle collection efficiencies could be examined at airstream velocities simulating typical indoor and outdoor air velocities, including indoor occupational settings (Baldwin and Maynard, 1998; Górny et al., 2002). Also, the setup allowed for the simultaneous investigation of collection efficiencies of multiple particle sizes.

A 25 mL slurry of A2 fine grade Arizona Road Dust (ARD; Powder Technology Inc., Arden Hills, MN) suspended in Milli-Q water ($8x10^{-3}$ g/mL; EMD Millipore Corporation, Milli-Q Direct 8, Billerica, MA) was aerosolized using a 3-Jet Collison Nebulizer (Q_C = 5 L/min, 20 psig; Mesa Labs, Inc., Lakewood, CO). The slurry was prepared by suspending 0.4 g of ARD in 50 mL Milli-Q water, hand shaking the mixture for 30 seconds, followed by sonication in an ultrasonic bath (Branson 8800 ultrasonic cleaner, Branson Ultrasonics, Danbury, CT) for 10 minutes. This preparation procedure ensured the slurry was sufficiently dissolved/mixed. As a result, the particle number size distribution produced by nebulization was repeatable across tests, including after prolonged use of the same suspension as well as whenever fresh suspension needed to be made (Figure A-1). Fresh slurry suspension (25 mL) was replaced in the nebulizer for each new film material tested, for different test runs using the same material and at least every 45 minutes. ARD was chosen to produce test particles because the resulting aerosol ranged in size from 0.014 μm to 6 μm covering the typical sizes of viruses, bacteria, and fungi (Reponen et al., 2011). For more details on the ARD's airborne particle number size distribution, see supplemental material and Figure A-1.

The aerosolized ARD particles were then carried through the wind tunnel test chamber by dry, filtered dilution air (Q_D = 30 L/min). The particles passed through a removable charge neutralizing section that either had an aluminum tube containing four 25.4 x 76.2 mm 500 μCi Po-210 ionizing units (2 mCi total; Amstat Industries, Glenview, IL) or an empty aluminum tube of the same dimensions. After passing the neutralizer, test particles were pushed into a test section where they were subject to collection by different polarized ferroelectric materials and controls.

The removable material test section shown in Figure 2-2B consisted of four square metal tubes (each 484 mm^2) fused together using a two part epoxy cold weld system (J-B KwikWeld, J-B Weld Co., Sulphur Springs, TX). These metal tubes were attached to a coupling for detaching/attaching the material test section from the rest of the sampling chamber using a rubber gasket. The entire test section was airtight so that the Q_{C+D} airflow with test particles would pass through the four metal tubes. Each tube contained a 3D-printed lattice spacer made of nylon (Figure 2-2C) holding parallel sheets of the test material. The use of exchangeable lattices allowed for easy testing of different materials and different air channel spacing between sheets of the same material. More pictures of the lattices are presented in Figure A-2.

2.4.2 Ferroelectric Films and Control Materials

Table 2-1 describes the tested polarized ferroelectric films and control materials. The two polarized polymer film types included PVDF $(CH2CF2)_n$- and a PVDF copolymer with trifluoroethylene (77% PVDF, 23% trifluorethylene). These polymer films are ferroelectrics – films in which the direction of permanent electric polarization can be switched (Nalwa, 1995). During manufacturing, these films were polarized by application of an external electric field. Once polarized, one side of the film has a negative electric field at the surface and the other side a positive electric field at the surface (Nalwa, 1995) until/unless the film is heated above its Curie temperature (Wang et al., 1988). It is my hypothesis that this permanent electric field can be used to significantly enhance capture of bioaerosols via electrostatic attraction.

Uniaxially oriented PVDF is a highly crystalline polymer with strong ferroelectric properties and a Curie temperature of about 167°C (Nalwa, 1995). Because it is

uniaxially oriented, the film will begin to lose its polarization if it is heated above 100°C. This means that the film is permanently polarized for typical environmental sampling purposes. The copolymer I used also exhibits strong ferroelectric properties with a Curie temperature of about 120°C, but does not need to be oriented before poling as it directly crystallizes into its most ferroelectric crystal form (Nalwa, 1995).

The stainless steel was included as a control to obtain a baseline of test particle deposition (loss) in the lattices compared to the tested films. The other control materials included two types of non-ferroelectric polymer films, PVC and PFA. PVC was chosen because it could be obtained in a tape form and be easily used to insulate the stainless steel metal to make it a sturdy, non-warping base to which all other test materials would be applied. This allowed the test materials to lie flat during testing in the spacing lattices. Consequently, this PVC-coated metal will be termed as the base (metal + PVC). For photographs of this base and spacing lattices, see figures A-2 and A-3. PFA was chosen as another control because, like PVDF and the copolymer, it is also a polar, fluorocarbon-based polymer and could be obtained as a thin film.

2.4.3 Preparation of Test Materials

All of the tested film materials and controls were prepared in the same way prior to testing. Gloves were worn during all experiments to prevent contamination of the film surfaces with skin oils. The film materials were cut to size (20 x 120 mm) with sharp, clean scissors, wiped with 70% ethanol to remove surface contaminants, and dipped into autoclaved Milli-Q water to remove ethanol residue. The base (metal + PVC) was cleaned with the same procedure after wrapping the metal with the PVC. The total PVC surface area was slightly larger than the other film types, but was the same as the metal's

(20 x 155 mm) (Figure A-2). All materials were allowed to dry overnight on a drying rack in a laminar flow hood (NuAire Biological Safety Cabinet, class II, type A2, Plymouth, MN), and then assembled inside the hood to minimize potential surface contamination. The flat pieces of test materials (films) were affixed with small pieces of double-sided tape to both sides of the base (metal + PVC) using less than 200 mm^2 of double-sided tape per piece of film. The bases with films were inserted into the lattices and formed horizontally oriented rows of films with fixed air channel widths between the rows (similar to a parallel plate microchannel setup). Depending on the experiment, lattices with two, three or four channels were used (Figure A-3). The polarized ferroelectric films were placed so that the positive polarization side of one film faced the negative polarization side of the other film across each air channel. For consistency, the positive polarization sides of the films were positioned to face upward except where noted below. Thus, during the experiments, air with test particles passed through the electric field present inside the channels formed by these test materials.

2.4.4 Film and Control Collection Efficiency Tests

As a conservative approach to investigate the practicality of using polarized, ferroelectric polymer films in a field deployable passive bioaerosol sampler, the ARD aerosol was charge neutralized because most ambient aerosol particles eventually exhibit Boltzmann equilibrium charge distribution (Hinds, 1982). The air velocity at entry into each metal tube of the film test section was about 0.35 m/s, which simulates typical indoor air conditions, including occupational environments (Baldwin and Maynard, 1998; Górny et al., 2002).

The collection efficiency of each test material was determined by measuring ARD particle size distributions by number upstream of the test section and downstream of each metal tube using an isokinetic port and copper probe, respectively. The combination of Scanning Mobility Particle Sizer Spectrometer (SMPS; model 3080 with Differential Mobility Analyzer 3081; TSI Inc., Shoreview, MN) and Aerodynamic Particle Sizer Spectrometer (APS; model 3321; TSI Inc.) measured particle number size distribution. The net aerosol charge was determined by an Aerosol Electrometer (Model 3068B; TSI Inc.). The viral, bacterial and fungal particle size ranges for these experiments were designated based on the size bins of the SMPS and APS and typical sizes of these bioaerosols: 0.01 to 0.25 μm for virus-size, 0.26 to 1.29 μm for bacteria-size, and 1.38 to 5.05 μm for fungi-size particles. Based on the air velocity and probe diameter, the isoaxial upstream inlet efficiency for sampling all size ranges of ARD was ~1.00.

As indicated in Table 2-1, the test materials had different thicknesses, and this affected the air channel width and, consequently, air velocity through the air channels. This was corrected for each setup by calculating the resultant air exit velocities and the isoaxial inlet efficiency for a horizontal sharp-edged inlet (aspiration + transmission efficiency) using equation 10-7 from Hinds (1982) (Durham and Lundgren, 1980). A fixed 2.25 ± 0.20 mm air channel width was used to test film materials. Preliminary investigations were performed to determine the air channel width which provided optimized particle capture for one type of poled, ferroelectric film (PVDF). These preliminary investigations were done by beginning with 10 mm wide air channels, and narrowing the air channels in 2 mm increments to determine differences in particle collection efficiencies (data not shown). Upon observation that air channel widths less

than about 6 mm resulted in differences in particle collection efficiencies, incremental air channel widths were investigated (more details below in section 2.4.5). The optimal air channel width was found to be 2.25 mm. The air channel width of 2.25 mm was then used to test the poled, ferroelectric films versus control materials. For consistency, the same air channel width was used for the experiments to compare particle collection efficiencies across film types.

With an air channel width of 2.25 mm, the exit airstream velocity was 1.00 ± 0.14 m/s across all tested materials. Since anisokinetic sampling leads to under or overestimation of the number of large particles in a sample (Hinds, 1982), downstream isoaxial inlet efficiency was determined for the midpoint diameter for each particle size range: 0.13 μm, 0.75 μm, and 3.22 μm for viral, bacterial and fungal-sized particles, respectively. The calculated inlet efficiencies ranged from 1.13 to 1.22 and were used as correction factors in my calculations.

For each test run, at least three rounds of upstream and downstream measurements were performed. Here, particle concentration downstream of each of the four metal tubes was measured before starting the next round of upstream sampling. As there were four tubes and each could hold a spacing lattice, two tubes contained lattices with a polarized, ferroelectric film material while the other two held the base (metal + PVC) as a control; the base (metal + PVC) was present for all repeats to estimate experimental variability across repeats. The tubes were sampled in random order. At least three repeats were done for each tested film type and control. This resulted in at least 18 upstream/downstream measurements per film material and control.

The indoor temperature throughout the experiments was 20-25 °C, and relative humidity stayed consistent at about 20-22%. The entire experimental setup was located in the laminar flow hood to minimize background particle interference. All tubing was conductive and as short as possible to minimize particle loss. Similarly, all chamber components were metal except where noted above in the description of Figure 2-2. The coefficient of variation of measured ARD particle concentration through the four tubes was 1.18%, 0.37% and 1.74% for the viral, bacterial and fungal sized particles, respectively, indicating uniform particle concentration across the chamber. The net charge for the ARD aerosol was indicated as current by the electrometer, and its reading was (-)37.3 ± 2.71 fA with ionizers present in the test chamber (Figure 2-2).

2.4.5 Testing of Collection Efficiency as a Function of Air Channel Width

The best-performing ferroelectric polymer from the tests above (section 2.3.4) was advanced to material spacing tests. The goal here was to investigate how the different air channel widths between the polymer films would affect ARD particle collection efficiency and then to select the optimum channel width for a sampler prototype. The experimental procedure was the same as that described above (sections 2.3.1 to 2.3.4) with two main differences. First, different sized spacing lattices were used to test air channel widths of 1.00 ± 0.05, 2.25 ± 0.20, 4.25 ± 0.20, and 6.25 ± 0.20 mm. Air channel widths were measured by a Vernier caliper (model #147, General Tools and Instruments, New York, NY). Preliminary tests suggested that this was the range of air channel widths where a difference would emerge in particle collection efficiencies across the tested films and controls. Since the total cross-sectional areas of the metal tubes which held the lattices (Figure 2-2B) were fixed, each lattice could accommodate fewer

channels once the air channel width increased. To account for this difference, the average collection efficiencies were corrected for the total surface area (number of channels in the setup) of the tested film material (for photographs of the lattices, see figure A-3). Second, experiments with both charge neutralized and non-charge neutralized ARD were performed. To get non-charge neutralized ARD, the ionizers were removed from the test chamber (Figure 2-2), which resulted in a net Aerosol Electrometer current for the ARD aerosol of (-)371.6 ± 128.8 fA.

Again, due to differences in film thickness and the differences in exit air velocity, correction factors were applied based on downstream isoaxial inlet efficiency. The downstream exit air velocities for the tested air channel widths were: 2.14 ± 0.09 m/s for width of 1.00 mm, 0.99 ± 0.02 m/s for 2.25 mm width, 0.68 ± 0.01 m/s for 4.25 mm width, and 0.68 ± 0.004 m/s for 6.25 mm width. These airstream velocities represent a range simulating typical indoor and outdoor conditions, including occupational environments (Baldwin and Maynard, 1998; Górny et al., 2002).

During each test run, all four lattices with different channel widths were loaded with the same test material and tested simultaneously (one lattice per tube). The placement of lattices into each of the four metal tubes was randomized. Each test run consisted of three repeated upstream/downstream measurements per lattice and it was repeated at least four times. Therefore, there were at least 12 repeated upstream to downstream measurement pairs per film material and different air channel width.

2.4.6 Collection and Deposition of Polystyrene Latex (PSL) Test Particles

The goal of these experiments was to determine collection efficiency and deposition location of PSL particles when using the ferroelectric film selected above

(sections 2.3.4 and 2.3.5). Airborne fluorescent PSL particles with 2 μm and 5 μm (G0200 and G0500, Thermo Fisher Scientific Inc., Waltham, MA) diameters were used, because they are within the main size ranges of interest for bioaerosol particles (Reponen et al., 2011). Second, these particles are fluorescent and large enough to allow for direct observation by microscope on the film surface where they deposited. Experiments with each PSL particle type were performed separately, but their aerosolized average particle mass concentration per test run was similar: 2.41 to 4.17 x 10^{-5} μg/cm^3.

Only lattices with 2.25 mm air channel width were used. Two diagonal lattices were loaded with clean bases and the same ferroelectric film type that was advanced to the air channel width tests. One of these lattices had the positive polarization side of the film facing upwards while the other had the negative polarization side of the film facing upwards. The other two diagonal lattices held clean base controls (metal + PVC). The experimental procedure was the same as described above (section 2.3.4), except collection efficiency was determined using both charge neutralized and non-charge neutralized 2 and 5 μm PSL. The collection of neutralized 2 and 5 μm PSL was performed sequentially onto the same films, and then clean films and control bases were used to collect non-charge neutralized 2 and 5 μm PSL. Since each lattice holds four film bases (Figure A-3), the setup resulted in eight ferroelectric film bases and eight controls (metal + PVC) for neutralized PSL tests, and the same number for non-charge neutralized PSL tests. Each test run consisted of three upstream to downstream measurement pairs, and two test runs were completed giving six repeated upstream to downstream measurements.

The number of PSL particles of each size deposited across the upward facing surfaces of the test films were counted using an epifluorescence microscope (Zeiss, Axio Imager.A1, Thornwood, NY) using the 20x objective and the reflected light setting. The films were marked by ink to have four equally sized (20 x 20 mm), square shaped quadrants (Q1-Q4) with Q1 being the front quadrant, i.e., entry point (Figure A-4). Twenty random microscope view fields were counted per quadrant. The areas of the film where the double-sided tape was located to affix the film to the base were not investigated. Thus, 80 view fields were analyzed per test film representing about 67% of the total film surface. The total number of PSL per quadrant was determined based on the view field area as described elsewhere (An et al., 2006).

The metal could not be used as a control here because of its reflective surface. So, the PVC-coated base was used instead since the PVC tape was matte black. The polarized, ferroelectric films were transparent, and so they also had a matte black background once affixed to the base (metal + PVC).

2.4.7 Particle Extraction Tests

Following microscope counting of PSL particles, I investigated extraction efficiency of captured particles from the ferroelectric polymer film surface using water-based ultrasonic agitation. Each of the bases was put into a 50 mL glass tube filled with autoclaved Milli-Q water. The tubes were covered with parafilm, vortex mixed for two minutes, then sonicated in an ultrasonic bath for either 8 or 40 minutes. This procedure was used as it was previously suggested to be effective and time efficient at removing captured microorganisms from filter material (Burton et al., 2005). Detergent was added to the ultrasonic bath water, but not to the individual glass tubes, to improve

ultrasonication-cleaning efficacy by reducing water surface tension and to prevent microbial contamination of the water (10 g detergent/L water, Alconox Detergent Powder, White Plains, NY). After washing procedures, the bases were allowed to dry completely and then the PSL remaining on the films were recounted in the same manner as conducted in section 2.3.6 to compare pre and post washing numbers on the films surfaces.

2.4.8 Statistical Analyses

Residual plots assessed statistical model assumptions for normality and homogeneity of variance. Due to unequal residual variances for some datasets, Welch's one-way analysis of variance (ANOVA) was performed to compare average particle collection efficiencies across different investigated film types and controls followed by Games-Howell multiple comparisons of means. The Welch procedure is generally applicable for simple comparisons (Oehlert, 2000). Individual samples t-tests were performed assuming unequal variances and two-tailed significance to compare the performance of polarized, ferroelectric polymer film to control (metal or PVC) under the same test conditions for the material spacing tests, PSL collection efficiency, and PSL extraction efficiency. Univariate general linear model (GLM) was also run to investigate complex interactions between particle size and test conditions when investigating the collection efficiencies for PSL particles. All analyses were performed using SPSS Statistics Premium Edition, v23 (IBM Corporation, 2015) with $\alpha = 0.05$.

2.5 Results and Discussion

Figure 2-3 shows the particle collection efficiencies (mean ± 1SD) across the different bioaerosol particle size ranges produced by the ARD for ferroelectric polymer

films and controls when using the same air channel width (2.25 mm). The final dataset was comprised of the following number of upstream to downstream sample measurement pairs per film type and control: lattice only (n=32), metal control (n=24), PVC control (n=136), PFA control (n=18), PVDF (n=18) and copolymer (n=9). The copolymer film I used was likely of low molecular weight which made it very brittle and difficult to handle without tearing; therefore, only nine repeat measurements were taken. Due to its fragility, the material was deemed unsuitable for advancement to the next tests.

One-way ANOVA for differences in particle collection efficiencies as a function of particle size range (virus, bacterial and fungal-sized particles) showed no statistically significant differences (Welch's $F(2, 133.292) = 1.049$, $p = 0.353$). As there was no significant effect of particle size on collection efficiency, the average collection efficiencies for all particle size ranges were pooled and investigated as a function of tested materials: two polarized, ferroelectric polymer film types and four controls. One way ANOVA showed a statistically significant effect of test material on average collection efficiencies (Welch's $F(5, 51.087) = 348.201$, $p < 0.001$).

Games-Howell posthoc test showed that the PVC and PFA controls and both polarized polymers had statistically significantly greater collection efficiency than the empty spacing lattice and lattice with metal control ($p < 0.001$). The average total collection efficiencies of the empty spacing lattice and metal control were $1.68 \pm 2.60\%$ and $5.61 \pm 6.17\%$, respectively. The average particle collection efficiencies for the PVC ($23.80 \pm 11.07\%$) and PFA ($23.89 \pm 6.90\%$) control films were not statistically significantly different ($p > 0.05$). The polarized, ferroelectric polymers, PVDF and copolymer, had average particle collection efficiencies of $29.59 \pm 4.67\%$ and $32.88 \pm$

1.99%, respectively, and were statistically significantly greater than all control types ($p < 0.05$), but were not significantly different from each other.

The PVC and PFA films increased the average particle collection over the metal control by 18.23 ± 0.06% (on the absolute scale). This may be due to the presence of a surface charge on these insulating materials that enabled the capture of those weakly charged particles that traveled close to the film. Previous research investigating submicron particle flow through parallel-plate microchannels suggests that particle flux to the walls of channels is strongly influenced by attractive surface interactions between oppositely charged particles and channel surfaces; these interactions include diffusion and electrical double-layer forces (Adamczyk and Van De Ven, 1981; Bowen et al., 1975; Bowen and Epstein, 1979). However, accumulation of surface charge on these materials is a stochastic process and cannot be relied on for continuous bioaerosol sampling.

The use of the polarized, ferroelectric polymers increased particle collection efficiency over the PVC and PFA control films by an additional 13.00 ± 2.33% (on absolute scale) resulting in an average collection efficiency for ferroelectric films of 30.69 ± 4.23%.This increased collection efficiency is due to the effect of film polarization. Electrets have been previously designed and successfully used for passive aerosol sampling (Brown et al., 1996; Brown et al., 1995; Brown et al., 1994a, b; Burdett and Bard, 2007; Thorpe et al., 1999). These electrets have been reported as being permanently polarized (Brown et al., 1994b; Seethapathy et al., 2008), but this is incorrect for corona poled polypropylene material like that described by Brown et al. (1994a). Corona poled polypropylene is well known for its ability to store electrical

charge and can have a remanent electrical field-induced polarization; however, as this polarization results from charge injection, the polarization will continuously and rapidly fade throughout use as the charge is neutralized by captured particles (Burdett and Bard, 2007; Zhang et al., 2012) or slowly over time if the films are stored for use.

In contrast, PVDF and its copolymers with TrFE can maintain their polarization until heated above their Curie temperatures. For passive bioaerosol sampling, this may provide more predictable, sustainable collection efficiency throughout use in the field over long-term sampling durations. As described by Brown et al. (1994b), electrets previously designed for passive aerosol sampling placed a conducting grid close to the surface of the electret-forming material as dust capture requires an electric field external to the electret. On the other hand, for polarized ferroelectric films, like PVDF, one side of the polarized film has a positive electric field associated with the film surface and the other side a negative electric field. Bringing the oppositely polarized sides of the film layers close enough together will result in the continuation of a constant electric field from one film to the other across the air channel(s).

Thus, the next goal of this research was to investigate the particle collection efficiency of paired layers of ferroelectric film as a function of air channel width. Since PVDF film showed the highest collection efficiency and the copolymer I obtained was not suitable due to its fragility, PVDF was advanced to the next round of testing. Figure 2-4 illustrates the particle collection efficiencies (mean ± 1SD) for neutralized and non-charge neutralized ARD at air channel widths ranging from 1.00 to 6.25 mm for PVDF and the metal control. An independent samples t-test showed no statistically significant effect of ARD charge state on collection efficiencies measured across air channel widths

for the PVDF and metal ($t(169.692) = 0.82$, $p = 0.935$). Therefore, to increase statistical power, collection efficiencies of the neutralized and non-charge neutralized ARD were pooled to represent an aerosol population with particles carrying both high and low electrical charge. For environmental sampling, such an aerosol population would be representative of freshly aerosolized and aged particle mix. Once the collection efficiency data from neutralized and non-charge neutralized ARD were pooled, the total number of upstream to downstream repeated measurement pairs were 96 and 144 for the PVDF and metal, respectively. For PVDF, there were 24 repeats per each air channel width. For metal control, there were 60, 36, 24 and 24 repeats at 1.00, 2.25, 4.25 and 6.25 mm air channel widths, respectively. At the smaller channel widths (i.e. ≤ 2.25 mm), higher turbulence was expected, and so a higher number of repeats were performed to improve estimates of variability for the control.

At each channel width, t-tests were performed to compare the collection efficiencies of the PVDF and metal control. PVDF resulted in a statistically significantly greater particle collection efficiency than metal at 2.25 mm channel width ($t(46) = 2.013$, $p < 0.001$) and 4.25 mm width ($t(38) = 2.024$, $p = 0.02$), but not at widths of 1.00 mm ($t(26) = 2.056$, $p = 0.53$) or 6.25 mm ($t(40) = 2.021$, $p = 0.18$). As per Welch's ANOVA, channel width was a statistically significant factor for collection efficiency of ARD by the PVDF (Welch's $F(3, 50.453) = 40.094$, $p < 0.001$). Games-Howell post hoc test revealed that average collection efficiencies obtained when using both the 1.00 and 2.25 mm air channel widths were statistically significantly greater than those using the 4.25 and 6.25 mm widths ($p < 0.05$), but were not significantly different from each other ($p > 0.05$).

Thus, it appears that the collection efficiency of PVDF is statistically significantly greater between about 2.25 and 4.25 mm air channel width compared to the metal control. At widths ≥ 6.25 mm, the strength of electric field created between the parallel PVDF films is no longer sufficient to provide a significant improvement in particle collection efficiency due to gravity and surface effects. At a distance of 1.00 mm, the collection efficiencies of the PVDF and metal were again not statistically significantly different. Previous research suggests that particle flux will increase as the air channel's surface boundary layer becomes comparable to distances over which attractive surface interactions dominate (Adamczyk and Van De Ven, 1981). Thus, for the air channel width of 1.00 mm, electrostatic attraction does not provide enhanced collection over micro-turbulence and particle-surface interactions. In addition, the 1.00 mm air channel width restricts airflow and is not practical for passive sampling where free air movement between the layers of PVDF films is essential. Based on data in Figure 2-4, it is apparent that PVDF films separated by an air channel of 2.25 mm perform the best and this width was used in further tests.

Figure 2-5 shows the collection efficiencies (mean ± 1SD) of neutralized and non-charge neutralized PSL test particles for PVDF versus the PVC control. (As described in Methods, PVC was used to insulate the metal base and was chosen as the control in these experiments since it provided a matte black background for microscopy). As per selection above (section 2.3.5), air channel width of 2.25 mm was used for experiments in Figure 2-5. The collection efficiencies of the PVDF and PVC were compared in four distinct tests: charge neutralized and non-charge neutralized PSL particles of 2 and 5 μm. There

were six total repeated upstream to downstream measurements used to compute collection efficiencies for each of the four test types.

Individual t-tests were used to assess differences in PVDF and PVC performance for each particle type. For charge-neutralized 2 μm PSL, the collection efficiencies of the PVC and PVDF were not statistically significantly different ($t(10) = 1.391$, $p = 0.194$). For non-charge neutralized 2 μm PSL as well as both neutralized and non-charge neutralized 5 μm PSL, the PVDF collection efficiency was statistically significantly greater than PVC's: $t(10) = 3.451$, $p < 0.05$, $t(10) = 3.585$, $p < 0.05$, and $t(10) = 5.796$, $p < 0.001$, respectively. A univariate GLM was run to assess prediction of particle collection efficiency including terms for PSL size (2 and 5 μm), PSL charge state (neutralized and non-neutralized), and film type (PVC and PVDF). The overall corrected model was statistically significant ($F(7)=53.871$, $p < 0.001$, $R^2 = 0.904$, adjusted $R^2 = 0.887$). Significant predictor terms included: PSL size ($F(1)=330.544$, $p < 0.001$), film type ($F(1)=33.576$, $p < 0.001$) and the interaction term between PSL size and PSL charge state ($F(1)=7.451$, $p < 0.05$).

These results indicate, as hypothesized, that polarized, ferroelectric PVDF films significantly enhanced particle collection efficiency when the test particles carried a higher electrostatic charge. Under Boltzmann charge equilibrium, larger particles tend to carry higher charge (Hinds, 1982). Therefore, even under Boltzmann charge equilibrium, the 5 μm PSL particles are expected, on average, to carry a higher number of charges per particle. Furthermore, a higher number of non-charge neutralized 5 μm PSL deposited on the positive polarization side of the PVDF near the point of entry into the film lattice

(Figure A-5). This further supports the conclusion that the PVDF films enhanced the collection of particles with opposite polarity to the film surface.

Another factor that has likely affected the results was the capture of particles by the 3D printed lattices themselves. Preliminary tests performed with 1 μm PSL test particles indicated that the loss of non-charge neutralized PSL when flowing through the empty spacing lattices was as high as 55% (data not shown). This result suggests that the 3D print material may be a candidate for producing the field deployable passive bioaerosol sampler because it enhances capture of charged particles in addition to their capture by the PVDF films. However, for this approach to be feasible in field sampling, the lattice has to be designed and integrated with the PVDF film in a way that allows for convenient and simple particle extraction from both the film and the film holder (lattice).

The extraction efficiencies (mean ± 1SD) of the PSL test particles deposited on the PVDF and PVC (control) films using liquid-based ultrasonic agitation are presented in Figure 2-6. As per t-test, the average PSL extraction efficiencies were statistically significantly greater from PVDF than from PVC for all tested conditions: 2 and 5 μm PSL and 8 and 40 minute sonication times. For 2 μm PSL and 8 and 40 minute sonication times, respectively: $t(14) = 2.881$, $p < 0.05$ and $t(14) = 2.827$, $p < 0.05$. For 5 μm PSL and 8 and 40 minute sonication times, respectively, the t-test results were: $t(14) = 3.181$, $p < 0.05$ and $t(14) = 5.006$, $p < 0.001$. Independent sample t-tests revealed no statistically significant difference between 8 and 40 minute sonication times across the two film types and particle sizes which suggests that particles deposited on PVDF films can be efficiently removed after 8 minutes ($t(61.389) = 1.351$, $p = 0.182$); thus, the shorter removal time can be used.

Data in Figure 2-6 show that up to 100% of particles deposited on PVDF films can be extracted for subsequent analyses. This is important for future sampler development as particle extraction efficiency affects representativeness of the sample. Moreover, this was achieved even with non-charge neutralized PSL particles, which carried a high net charge upon aerosolization (Figure 2-5) and would experience strong electrostatic attraction once deposited on the polarized PVDF films. However, even in this scenario, the particles were efficiently removed suggesting that collected biological particles can be effectively removed as well. The extraction efficiency of surface deposited microorganisms will be addressed in subsequent studies.

2.6 Conclusions

This research investigates the application of permanently polarized ferroelectric polymer films for passive bioaerosol sampling in the first stages of designing a sampler concept. A polarized, ferroelectric polymer film, PVDF, was found to provide statistically significantly greater collection efficiency than controls for particles exhibiting Boltzmann charge equilibrium ranging in size from 14 nm to 5 µm. This represents typical sizes of viral, bacterial and fungal particles (Reponen et al., 2011). The charged airborne particles are captured by the electrostatic field created between parallel, oppositely polarized surfaces of the ferroelectric polymer films when particles flow through air channels that are 2.25 to 4.25 mm wide. The range of air flow velocities used in these experiments (~0.35 to 2.16 m/s) represents typical air velocities in indoor (including occupational) and outdoor spaces (Baldwin and Maynard, 1998; Górny et al., 2002).

While electret materials have been previously used in passive aerosol sampling, these materials will lose their charge during use as charged particles deposit onto the collection surface (Burdett and Bard, 2007). In contrast, polarized PVDF films maintain permanent electric fields at typical environmental temperatures (up to 100 °C, where the polarization begins to decrease); thus, it is hypothesized that they can be applied for long-term bioaerosol sampling without degradation of their performance. Also, charged PSL particles deposited onto the PVDF film surface were removed from the films with 100% efficiency indicating minimal sample loss during post-sampling extraction.

PVDF will be advanced to the next round of passive bioaerosol sampler development. The next development stages will focus on prototyping and testing different field-deployable sampler designs under controlled laboratory conditions with different types of microorganisms. This chapter provides a conceptual framework for the passive bioaerosol sampler's design and development. Ultimately, this sampler will provide a new tool for bioaerosol research. Applying polarized, ferroelectric polymer films to passive bioaerosol sampling will expand possibilities for performing long-term sampling and exposure assessment to better understand how airborne biological particles influence health and the environment. Alternatively, these films can also be integrated with air moving devices (e.g. pumps, fans) as a type of electrostatic precipitator that does not need power for particle charging or collection; this would provide a low power option for active bioaerosol sampling without production of ozone.

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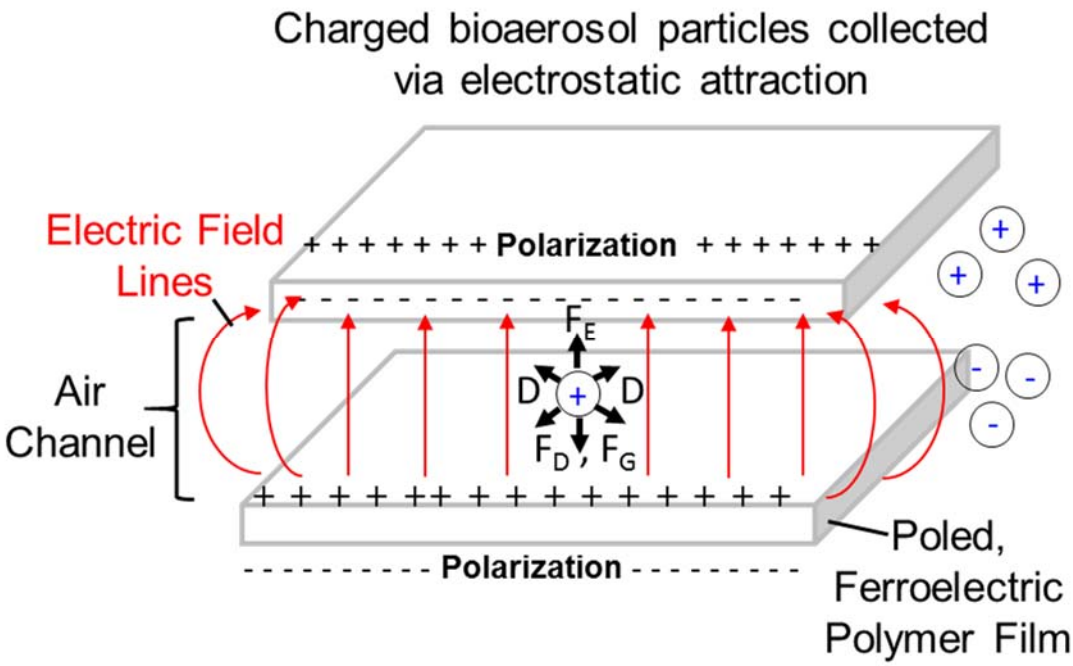

Figure 2-1.

The conceptual diagram for how parallel oriented layers of a ferroelectric polymer film may be used to electrostatically capture charged bioaerosol particles by a continuous electric field from one film surface to the other across an air channel. While there may be surface charge present on these films, film polarization results in an electric field associated with the film surfaces and so particle capture is not dependent upon film surface charge. The electric field lines of the sampler extend outward which increases the exposure of the sampler's electric field to airborne particles. Particles are attracted to and deposited onto the film's poled surface of opposite polarity to the charge on the particle. The forces and mechanisms are illustrated which influence particle capture for an imaginary positively charged particle between the sampler films. These include: electric force (F_E), drag force (F_D), gravitational force (F_G), and particle diffusion (D). Diffusion would be the same in all directions in the absence of a particle concentration gradient.

Table 2-1.

Ferroelectric Films and Control Materials			
Ferroelectric Film Materials and Controls	**Details**	**Material Thickness (μm)**	**Material Classification**
Poly(vinylidene fluoride) (PVDF)	Polarized, uncoated poly(vinylidene fluoride) homopolymer; uniaxially oriented (Kureha America LLC, New York, NY)	28	Polarized, ferroelectric polymer
PVD(TrFE) (copolymer)	Polarized, uncoated copolymer film; 77% PVDF, 23% trifluorethylene (Piezotech Arkema, Pierre-Benite Cedex, France)	20	Polarized, ferroelectric polymer
Metal	Stainless steel, non-warping (Model 10414, Westcott, Fairfield, CT)	500	Conductor (control)
Poly(vinyl chloride) tape (PVC)	Vinyl electrical tape (Model 30002653, Commercial Electric Inc., Wasilla, AK)	356	Insulator (control)
Perfluoroalkoxy alkane (PFA)	High temperature, high strength perfluoroalkoxy alkane; marketed under Teflon brand (Model 84955K22, McMaster-Carr, Princeton, NJ)	127	Insulator (control)

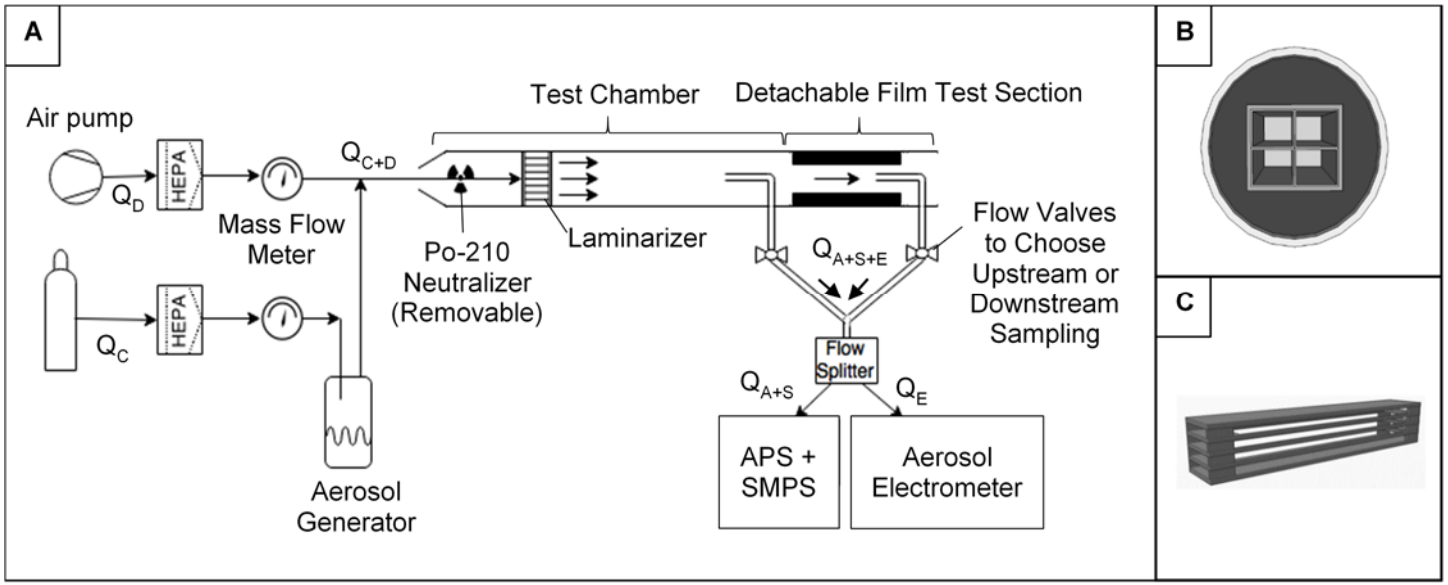

Figure 2-2.

Experimental wind tunnel setup for testing candidate film materials (2-1A). Front view of the detachable film test section (2-1B) and spacing lattices (2-1C) which insert into the square chambers of 2-1B to set air channel widths between parallel sheets of tested film materials. Photographs of these spacing lattices are illustrated in Figure A-3. The combination of Scanning Mobility Particle Sizer Spectrometer (SMPS; model 3080 with Differential Mobility Analyzer 3081; TSI Inc., Shoreview, MN) and Aerodynamic Particle Sizer Spectrometer (APS; model 3321; TSI Inc.) were used to measure particle number size distribution. Net aerosol charge was determined by an Aerosol Electrometer (Model 3068B; TSI Inc.).

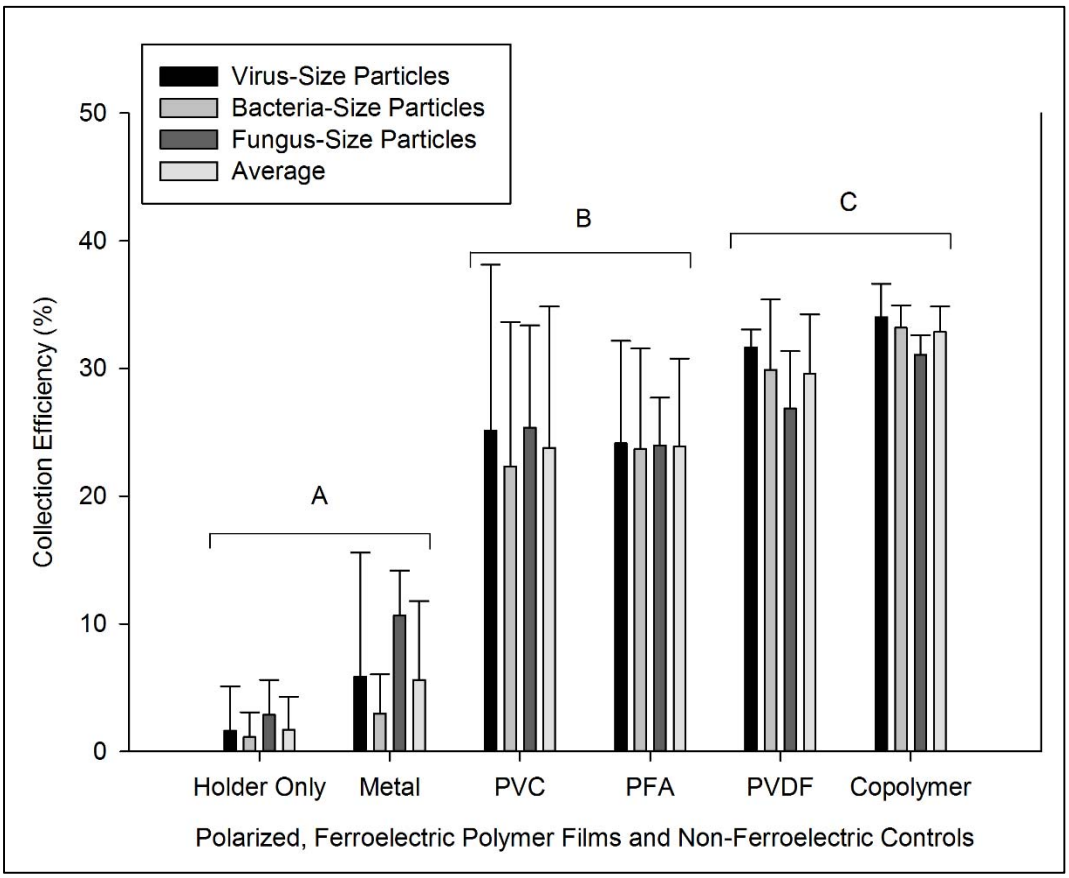

Figure 2-3.

Collection efficiencies (mean ± 1SD) of polarized, ferroelectric polymer films and controls across typical bioaerosol particle size ranges (viral, bacterial, and fungal-sized particles). All film testing was done using 2.25 mm air channel width between parallel layers of films. One way ANOVA revealed a statistically significant effect of film type on average particle collection efficiencies (Welch's $F(5, 51.087) = 348.201$, $p < 0.001$). According to Games-Howell post hoc test, groups A, B, and C are statistically significantly different for total particle collection ($p < 0.05$) while the film types within each group A-C are not statistically significantly different from each other ($p > 0.05$). As a conservative approach, test particles were charge neutralized for these experiments to exhibit Boltzmann charge equilibrium. The number of sample repeats were as follows:

empty lattice (holder only) (n=32), metal control (n=24), PVC control (n=136), PFA control (n=18), PVDF (n=18) and copolymer (n=9).

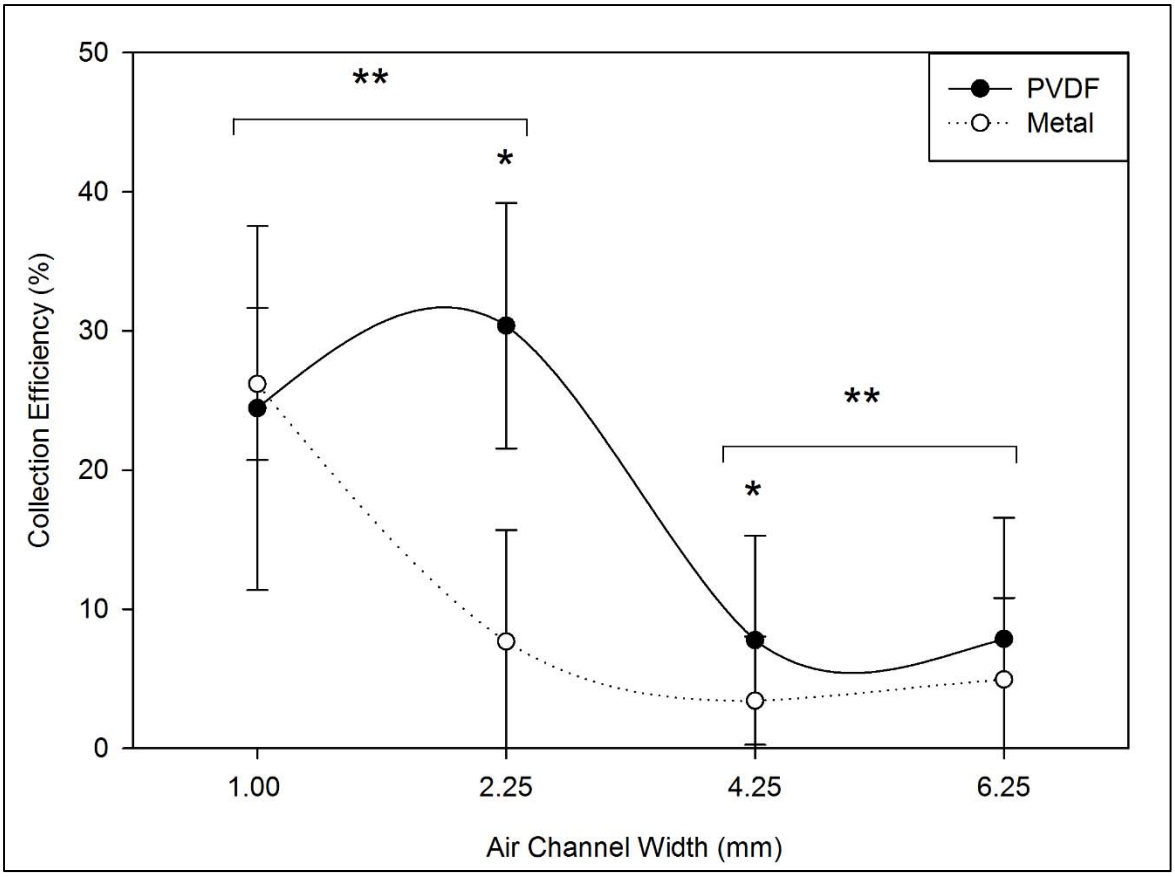

Figure 2-4.

Particle collection efficiencies (mean ± 1SD) of ARD for PVDF versus metal control using air channel widths ranging from 1.00 to 6.25 mm. A "*" indicates statistically significant difference between mean particle collection efficiency for PVDF versus metal control at a given air channel width ($p < 0.05$). Comparing the collection efficiencies of the PVDF across the four air channel widths, there was a statistically significant effect on average ARD collection efficiency (Welch's $F(3, 50.453) = 40.094$, $p < 0.001$). As shown by "**", Games-Howell post hoc test revealed that average collection efficiencies obtained when using both the 1.00 and 2.25 mm air channel widths were statistically significantly greater than using the 4.25 and 6.25 mm widths ($p < 0.05$), but were not significantly different from each other ($p > 0.05$). For PVDF, there were 24 repeats per

each air channel width. For metal control, there were 60, 36, 24 and 24 repeats at 1.00, 2.25, 4.25 and 6.25 mm air channel widths, respectively.

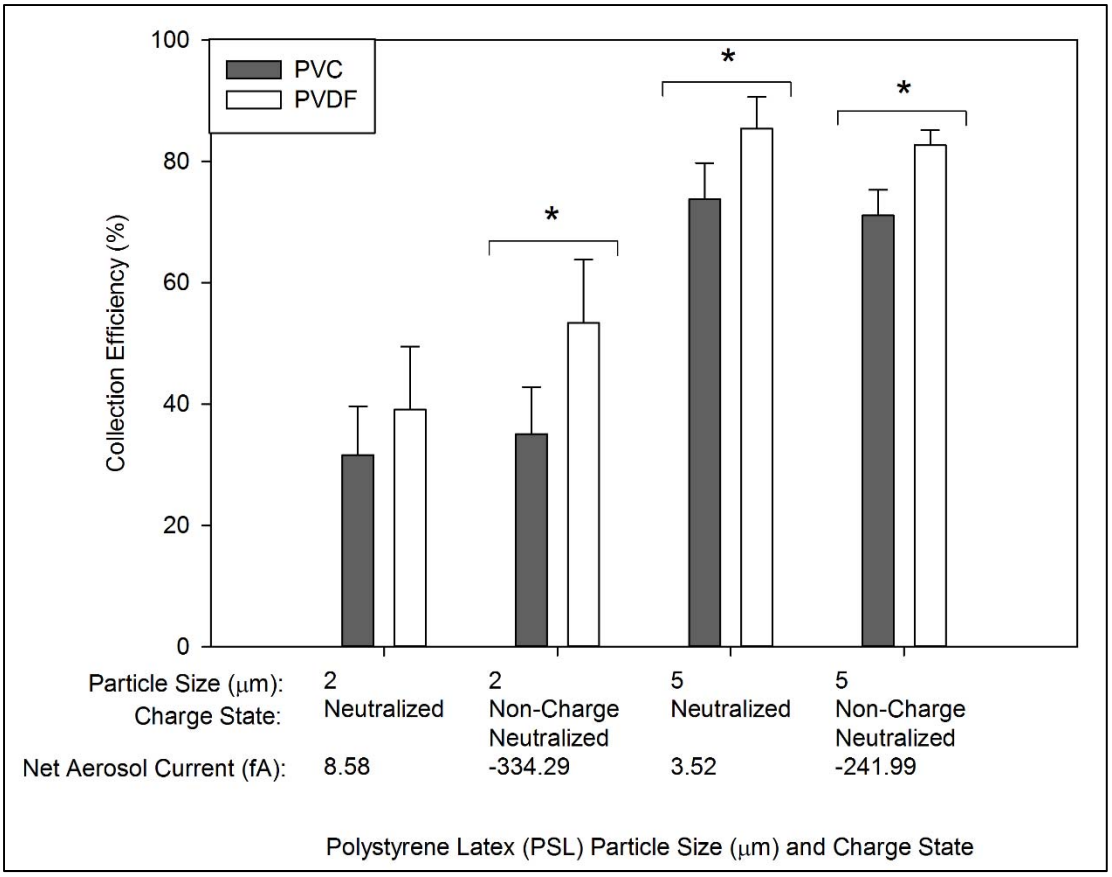

Figure 2-5.

Collection efficiency (mean ± 1SD) of neutralized and non-charge neutralized polystyrene latex (PSL) test particles (2 and 5 μm diameter) captured using either PVDF or PVC control. Capture of particles was done using 2.25 mm air channel width. A "*" indicates statistically significant difference between PVDF and PVC collection efficiency for a given test particle size and charge state. There were six total repeated upstream to downstream measurements used to compute collection efficiencies for each of the four test types.

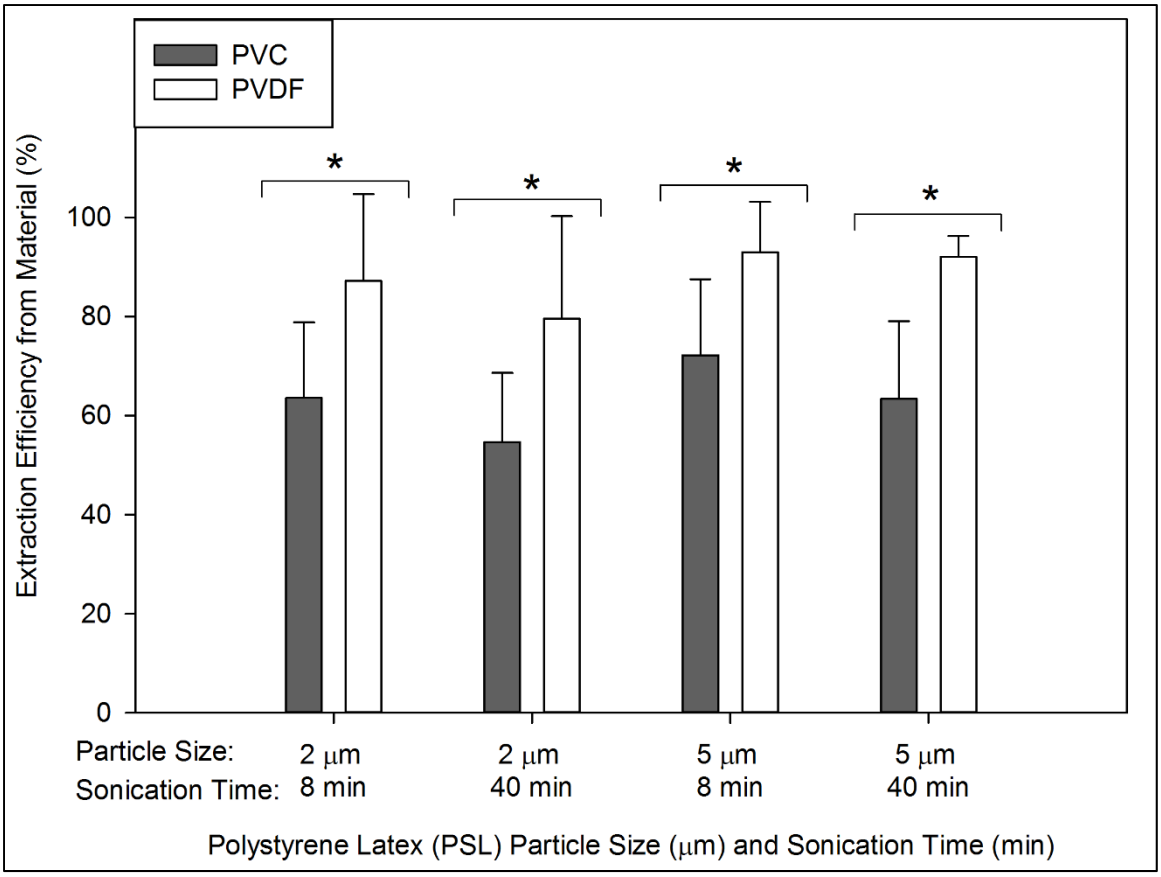

Figure 2-6.

Extraction efficiencies (mean ± 1SD) of polystyrene latex (PSL) test particles (2 and 5 μm) from PVDF and PVC control using two different ultrasonic bath times: 8 versus 40 minutes. A "*" indicates statistically significant difference between particle extraction efficiency from PVDF and PVC for a given particle size and ultrasonication time. Repeats numbers for these tests included eight ferroelectric film bases and eight controls (metal + PVC) for neutralized PSL extraction tests, and the same number for non-charge neutralized PSL extraction tests.

CHAPTER 3. PROTOTYPE TESTING OF PASSIVE BIOAEROSOL SAMPLER DESIGNS IN A COMPACT, CALM AIR SETTLING CHAMBER

Material in this chapter has been previously submitted for publication as:

Therkorn, J.; Thomas, N.; Calderon, L.; Scheinbeim J.; Mainelis, G. Prototype Testing of Passive Bioaerosol Sampler Designs in a Compact, Calm Air Settling Chamber. *Aerosol Science and Technology* **2016**, submitted.

3.1 Abstract

A new, passive bioaerosol sampler is needed to better estimate bioaerosol concentrations and exposures occurring over broad spatiotemporal scales. Here, I present testing of a passive bioaerosol sampler concept which uses polarized ferroelectric polymer film (poly(vinylidene fluoride)), (PVDF) incorporated into a 3D-printed film holder. The extraction efficiencies of spiked bacteria and fungal spores off PVDF, prototype film holder materials (3D-printed plastics), and controls (PTFE filters and electrostatic dust cloths) were investigated. Collection efficiencies of passive sampler prototypes were determined in a compact, calm air settling chamber relative to a passive filter when collecting a mixture of airborne bacteria. Two prototype sampler designs - lattice and spiral - and three film conditions for these designs – holders only (no film), unpoled PVDF film, and polarized (poled) PVDF film – were tested. 100% extraction efficiency of microorganisms from the PVDF film and 3D-print materials was achieved. The integration of poled PVDF film with the prototype designs provided a > six-fold increase in captured bacterial quantity compared to gravimetric settling onto a filter with similar projected surface area ($p < 0.05$). This improvement was achieved due to the polarization of the PVDF film. This study further refines the development of a field deployable passive

bioaerosol sampler using polarized ferroelectric polymer and a 3D-printed film holder. The spiral prototype design securely held the PVDF film with optimized air channel distances between the coiled, parallel layers of the film and was easy to assemble. Thus, this design will be advanced to the next stage – outdoor field testing.

3.2 Introduction

Bioaerosol consists of airborne particles of biological origin, like viruses, bacteria, fungi, pollen, and fragments or toxins produced from living organisms. Bioaerosol can affect human health through a range of activities, like infectivity, allergenicity, toxicity or other similar processes (Cox and Wathes, 1995). Current bioaerosol-related concerns broadly range from issues like the spread of antibiotic resistant genes from bacterial aerosol produced during wastewater treatment (Li et al., 2016) to impacts of climate change on production, release and dispersion of allergenic fungal spores and pollen grains (Hamaoui-Laguel et al., 2015; Sadyś et al., 2015).

Due to the wide ranging and significant environmental health impacts of bioaerosol particles, there needs to be more focus on developing new technologies for estimating bioaerosol exposures and investigating how bioaerosol interacts with the environment (Peccia et al., 2008). Currently, bioaerosols are typically collected via active sampling, e.g., using air movers; however, passive sampling (e.g., no air movers present) allows for sampling strategies that better represent bioaerosol exposures occurring over longer timeframes and across wider spatial scales (Cherrie and Aitken, 1999; Tovey et al., 2003; USEPA, 2016). Example applications include epidemiological and ecological research where a high number of cost-effective replicate samples are desired for exposure assessment (Adams et al., 2015). Hence, there is a need for more

development of passive bioaerosol sampling technology as there are currently very few options for passive bioaerosol sampling (Therkorn et al., 2016).

Some studies have evaluated the performance of different types of materials and approaches to directly collect settling biological particles, including use of agar settling plates, empty petri dishes and electrostatic dust cloths (Adams et al., 2015; Nasman et al., 1999; Noss et al., 2008; Sayer et al., 1972; Sayer et al., 1969; Tovey et al., 2003). Adams et al. (2015) suggests that smooth, non-porous materials, like hard plastics, will provide greater overall post-sampling detection of microbial material because it is difficult to extract collected particles from fibrous materials, like dust cloths, for post-sampling analyses. Thus, new passive bioaerosol sampler technology has to not only focus on optimizing particle collection efficiency across different size ranges, but also preservation of biological quality, sample handling and storage, and post-sampling analyses (Adams et al., 2015; Nasman et al., 1999; Reponen et al., 2011). When choosing a method for passive bioaerosol sampling, key factors include simplicity, practicality and the capability of the chosen method to detect and preserve the biological agents of interest (Tovey et al., 2003).

Therkorn et al. (2016) describe a new passive sampler concept where collection of settling biological particles is electrostatically enhanced by using parallel layers of permanently polarized ferroelectric polymer films (e.g., poled poly(vinylidene fluoride), or PVDF) with 2.25 mm wide air channels between the polymer layers. This passive bioaerosol sampler concept is of interest for further development because it was found to provide effective capture of particles in size ranges of interest for bioaerosol (0.014 – 5 μm), efficient post-sampling particle extraction, and flexibility in final sampler design

(Therkorn et al., 2016). Here, I develop this sampler concept further and compare bioaerosol collection efficiencies of different field-deployable prototype designs. The objectives for these prototype tests are to determine the design that maximizes collection efficiency for a mixture of bacterial aerosols and to investigate extraction efficiency of different types of microorganisms from the test materials, including the PVDF film and materials used to produce the film holders (3D-printed plastics). In order to accomplish these objectives, I modified a calm air settling chamber (Feather and Chen, 2003) as described below to test prototype designs using a more compact chamber model with a mixture of bacteria aerosolized into the system. This chapter presents the refinements for the passive bioaerosol sampler prior to field testing.

3.3 Materials and Methods

3.3.1 Microorganism Extraction Tests

3.3.1.1 Test Materials

Five test materials were compared for the extraction efficiency of spiked microorganisms from their surfaces. These materials included 1) one type of ferroelectric polymer film, polarized, uniaxially-oriented, uncoated, 28 μm thick poly(vinylidene fluoride) (PVDF) (Kureha America LLC, New York, NY). This material was used in early prototype designs (Therkorn et al., 2016). Two types of 3D-print materials (Shapeways, New York, NY) were included: 2) an acrylic (rigid opaque photopolymer, VeroWhitePlus (RGD835), Stratasys Ltd., Eden Prairie, MN) and 3) a nylon (polyamide 2200). These 3D-print materials were chosen because they are cost-effective, strong, flexible and print with high resolution, making them prime candidates for producing the passive bioaerosol sampler body prototypes. Since the sampler body, a 3D-printed film

holder, may also passively collect settling particles (Therkorn et al., 2016), the sampler should ideally allow for post-sampling particle extraction from both the films and the 3D-printed film holder – hence the reason for their inclusion in the tests. Finally, two types of control materials were included as well: 4) PTFE filters (Zefluor^{TM}, 25 mm diameter, 2 μm pore size, SKC Inc., Eighty Four, PA) as one control, and 5) electrostatic dust cloths (ESDs, Swiffer Brand, Procter & Gamble, Cincinnati, OH) as a second control. The control materials were included because they have been commonly used for active (PTFE filters) or passive (ESD) bioaerosol sampling and have been previously tested for biological particle extraction efficiency (Adams et al., 2015; Burton et al., 2005; Noss et al., 2010; Noss et al., 2008; Wang et al., 2001).

Together, the PVDF, 3D-print materials, PTFE filters and ESDs represent different textures of smooth/non-porous (PVDF and 3D-printed acrylic), smooth/porous (PTFE filters and 3D-printed nylon), and fibrous (ESDs) materials. The PVDF, PTFE filters and ESDs are hydrophobic.

3.3.1.2 Microorganisms

Extraction efficiencies of selected test materials were investigated for three different microorganisms, including two bacterial species, *Bacillus atrophaeus* (American Type Culture Collection Inc. (ATCC 49337), Rockville, MD) and *Pseudomonas fluorescens* (ATCC 13525), and fungal spores of *Penicillium chrysogenum* (ATCC 10135). These microbial species have been commonly used and recommended for bioaerosol research (Han et al., 2015). *B. atrophaeus* is a Gram-positive, representative hardy bacterium, and the *Bacillus* genus of bacteria are commonly found indoors and outdoors (Fang et al., 2007; Hospodsky et al., 2010; Li, 1999; Stanley et al.,

2008; Zhu et al., 2003). *P. fluorescens* is a Gram-negative bacterial species commonly used to represent sensitive bacteria in bioaerosol research in contrast to Gram-positive species (Seshadri et al., 2009; Stewart et al., 1995; Yao and Mainelis, 2006). *P. chrysogenum* is one of the most commonly found fungal species indoors in water-damaged buildings – a common scenario of concern for airborne mold exposure (Andersen et al., 2011). *B. atrophaeus*, *P. fluorescens,* and another fungus of the *Penicillium* genus (*P. melinii*) have also been previously investigated for extraction efficiency of different filter materials (Burton et al., 2005; Wang et al., 2001). The aerodynamic diameters of *B. atrophaeus* (vegetative cells), *P. fluorescens*, and *P. chrysogenum* are 0.8 μm, 0.8 μm and 2.8 μm, respectively (Madelin and Johnson, 1992; Qian et al., 1997; Willeke et al., 1996).

Active *B. atrophaeus* and *P. fluorescens* cultures were inoculated in nutrient broth (CM0001, Oxoid LTD., Basingstoke, Hampshire, England) and incubated at 30°C and 26°C, respectively, for 18 hours. After incubation, all cells were harvested from the broths by centrifugation for five minutes at 7000 rpm (BR-4 centrifuge, Jouan, DEC Inc., Lorton, VA). Cells were then washed three times by resuspending pellets in sterile Milli-Q water (EMD Millipore Corp., Milli-Q Direct 8, Billerica, MA) and repeating centrifugation. The final washed cells were resuspended in 10 mL sterile Milli-Q water. *P. chrysogenum* spores were streaked on malt extract agar (Difco, Becton, Dickinson and Co., Sparks, MD) and incubated at room temperature for seven days. After incubation, spores were harvested off the agar with sterile Milli-Q water and gentle scraping (Yao and Mainelis, 2006). The fungal spores were then washed by the same centrifugation technique as the bacterial cells and resuspended in 10 mL sterile Milli-Q water.

3.3.1.3 Test Procedure

A 100 µL aliquot of each microbial suspension was vortex mixed with 100 µL formalin (37% by weight, Fisher Scientific, Fair Lawn, NJ) and 800 µL Acridine Orange stain (0.1 g/L, Becton, Dickinson and Company, Sparks, MD), left in a dark environment for 1 hr, then serially diluted to count the initial suspension concentrations using an epifluorescence microscope and a 100x oil immersion objective (AX10, Carl Zeiss, Thornwood, NY). Stained cells were filtered through a black polycarbonate filter (GTMP, 0.2 µm, 25 mm, Merck Millipore Ltd., Cork, Ireland) and fixed onto a microscope slide. Then, at least 40 random field views were counted, averaged, and the total number of cells was estimated by multiplying the average count by 6125 to scale up to the entire filter size. For the initial suspension concentrations, the bacteria had ~10^9 cells/mL and fungi spores had ~10^7 spores/mL, respectively.

The test material pieces were of similar size. The PVDF and ESDs were cut into square pieces of 625 mm^2. The PTFE filters were 491 mm^2. The 3D-print material coupons were printed to be 525 mm^2. The materials were cleaned using the most rigorous methods that were appropriate for not damaging the materials. PVDF was wiped with 70% ethanol and the 3D-print materials were soaked in ethanol for one hour then vortex mixed in ethanol. This method was used for the 3D-print materials because they are not printed watertight and it was desirable to remove any contaminants from their interstitial space. The PVDF and 3D-print materials were then dipped into sterile Milli-Q water to remove ethanol residue. The ESDs and PTFE filters were autoclaved at 121°C for 25 minutes in sterilization pouches (Chex-all II instant sealing, Propper Manufacturing Co., Long Island City, NY). All materials were allowed to dry completely overnight in a

laminar flow hood (NuAire Biological Safety Cabinet, Class II, Type A2, Plymouth, MN).

The microbial suspensions were spiked onto the test materials to produce at least five repeat test material coupons per microorganism species. There were also three blanks included per test material for which sterile, Milli-Q water was spiked onto the material surface in the same manner. To spike bacteria, four 25 µL drops (100 µL total ≈ 10^8 bacteria) were applied onto each test material piece. To spike fungi, four 50 µL drops of the fungal suspension (200 µL total ≈ 10^6 to 10^7 spores) were applied onto each test material coupon since the initial concentration of the fungal spores was lower than bacteria. This method of spiking was used so that the microbes were spread out across the test material surface, the droplets would dry faster and it was easier to prevent smaller droplets from rolling off the material surface during application. All droplets were allowed to dry completely in the laminar flow hood for at least four hours.

After the droplets dried, the materials underwent the same extraction procedure to determine the extraction efficiencies of the microbes from the materials. Therkorn et al. (2016) investigated extraction efficiency of 2 and 5 µm polystyrene latex test particles from PVDF and determined that two minutes of vortexing followed by a short (eight minute) ultrasonic bath time can be used to remove these particles with up to 100% efficiency. Since time-efficient removal of collected microorganisms is desired in the final prototype sampler design, the same extraction procedure was tested in the present experiments with two procedural changes; first, a 10 minute ultrasonic bath time was used instead for convenience. Second, each test piece coupon was placed into a 50 mL conical centrifuge tube (Falcon Tubes, Corning Inc., Tewksbury, MA) containing 20 mL

sterile, Milli-Q water. It should be noted that, unlike similar previous studies (Burton et al., 2005; Wang et al., 2001), Tween was not included in the extraction fluid because it may cause membrane damage to collected microorganisms potentially introducing bias to microbial quantification post-sampling (Zhen et al., 2013). After extraction, a 100 µL aliquot was taken from each extraction suspension and stained/counted as described above. The average counts of the blanks were subtracted from the resultant totals for each material type.

3.3.2 Passive Bioaerosol Sampler Prototypes

Figure 3-1 illustrates the two models of passive bioaerosol sampler prototypes tested as holders for the PVDF film in the calm air settling chamber: a lattice design and a spiral design. PVDF film has been shown to significantly enhance capture of bioaerosol-sized particles (0.014 to 5µm) via electrostatic mechanism as the particles were carried through 2.25 mm wide air channels between parallel layers of the film (Therkorn et al., 2016). Thus, the prototype designs tested here maximized the number of parallel film layers with 2.25 mm wide air channels. These parallel film layers were set up so that the positive and negative polarization sides of the films would face each other across the air channels so as to form a continuous electric field between the films (Therkorn et al., 2016).

The overall dimensions of the two models were similar and the holders were designed to conveniently fit into standard 50 mL conical centrifuge tubes; the purchased tubes are pre-sterilized and provide a convenient and inexpensive container for sampler storage, transport and expedited particle extraction by the method described. The selected holder dimensions allow the prototypes to freely move inside the tube for vortex mixing

and ultrasonication when the tubes are filled with 40 mL sterile, Milli-Q water. The extraction volume of 40 mL is used as the minimum volume needed to fully submerge the samplers when in the 50 mL conical centrifuge tube.

For the lattice prototype, eight pieces of PVDF film of size 15 x 70 mm (8400 mm^2) were positioned in three parallel facing layers. The films were affixed to either side of the holder's four vertical supports with small pieces of double sided sticky tape. For the spiral prototype, one piece of PVDF film 130 x 70 mm (9100 mm^2) was threaded through the openings to form a spiral with multiple film layers with opposite sides of the film facing each other across the air channels between the threaded layers. No tape or adhesive was needed to hold the films in the spiral holder. After cutting the PVDF film to size with clean, sharp scissors and before assembling the film into the prototype holders, the PVDF and the prototype holders were cleaned as described above (section 3.3.1.3). The films were assembled into the prototype holders and allowed to dry overnight in the laminar flow hood.

Unpoled, un-oriented 25 µm thick PVDF film (Kynar brand, CS Hyde Co., Lake Villa, IL) was used as a control and prepared (cleaned and integrated with film holders) in the same way as the poled PVDF. This control is a simple fluorocarbon polymer film with the same surface area as test films, but without the properties of polarization and film orientation.

3.3.3 Calm Air Settling Chamber Design and Experimental Modifications

PVDF film has already been shown to capture particles electrostatically as they were moving at typical indoor and outdoor air velocities through the channels formed by the film (Therkorn et al., 2016). Therefore, the research presented here used a calm air

settling chamber to investigate collection efficiencies of field-deployable passive sampler prototypes using PVDF films. Such a chamber allows for the investigation of how sampler prototypes electrostatically capture particles already undergoing gravitational settling, i.e., the diversion of particles from their settling paths without forcing air through the film layers. Small, calm air settling chambers have been previously used to compare aerosol sampler performances (Kenny et al., 1999; Thorpe and Walsh, 2007). Additionally, evidence suggests that many indoor environments, including occupational and residential, typically have low wind speeds (less than about 0.3 m/s) (Baldwin and Maynard, 1998; Matthews et al., 1989) and settling chambers simulate these calm air conditions.

The calm air settling chamber described by Feather and Chen (2003) was modified to reduce its size in order to maximize time efficiency of performing multiple experimental repeats for aerosolized *B. atrophaeus* and *P. fluorescens* bacteria which have very slow settling velocities (~2.33×10^{-5} m/s) (Hinds, 1982; Reponen et al., 2011). Due to such low settling velocities and interference of Brownian motion, the second goal of the modifications was to improve mixing of bacteria in the chamber without disrupting the calm air conditions in the chamber.

Here I describe the specific settling chamber (Figure 3-2) design modifications; all other details are described elsewhere (Feather and Chen, 2003). A suspension of two bacterial species (20 mL) was aerosolized using a 3-Jet Collison nebulizer at Q_A = 5.40 L/min, 25 psig (Mesa Labs Inc., Butler, NJ). The aerosolized bacteria passed through a diffusion dryer (model 3062, TSI Inc., Shoreview, MN) and then entered into the calm air settling chamber which was constructed of a standard 5 gal. (18.9 liters) plastic pail

(Leaktite, Leominster, MA). Fresh bacterial suspension was used for each experimental repeat. The bacteria were aerosolized into the chamber for 60 min, and the inlet nozzle was rotated 60° every 20 min. The overall volume of the calm air chamber was ~2.35×10^4 cm^3 which reduces the overall chamber size by about half compared to that described by Feather and Chen (2003). The inside of the chamber was fully lined by conductive aluminum foil tape (3M, St. Paul, MN) to reduce particle loss to the inside chamber surface due to charge effects.

After the aerosolization, the system was left undisturbed to allow bacteria to completely settle for about five hours. To increase the homogeneity of the bacterial distribution presented to both tested prototypes and controls, a rotating platform was installed on the bottom of the chamber. The platform was 3 mm thick wood laser cut to a three leaf clover shape. The platform was then placed onto a high torque movement clock motor (The Clock Shoppe, Sycamore, IL) with rotational velocity of 1 rpm. The rotation was slow and non-continuous since the platform turned and stopped every second; this helped to ensure that no turbulence or net aerosol movement occurred in the sampling area of the chamber.

Bacterial suspension consisted of *B. atrophaeus* and *P. fluorescens* cells prepared as described above (section 3.3.1.2), but final washed cells were resuspended in 40 mL sterile, Milli-Q water, then mixed together to provide 80 mL of mixed *B. atrophaeus* and *P. fluorescens* bacteria. Bacteria were mixed together because it is a better simulation of typical environmental conditions where a variety of bacterial species are present.

For each experimental repeat, two prototype sampler designs and one reference 25 mm PTFE filter were placed onto a randomly chosen “leaf” of the three leaf clover

shaped platform. The reference PTFE filter was included so that collection efficiency results obtained for the prototypes could be normalized to (e.g. divided by) the number of bacteria that settled onto the reference filter, thus accounting for changes in bacterial concentration between experimental repeats. The number of bacteria extracted from the PTFE filters was adjusted for extraction efficiency less than 100% as determined from the microorganism extraction tests (Materials and Methods Section 3.2.1). The prototypes and reference filter were anchored to the platform for each test using a small piece of double-sided sticky tape. The reference filter, lattice and spiral prototypes had projected surface areas of 490.87 mm^2, 270 mm^2 and 380.13 mm^2, respectively.

After a five hour wait, the chamber was opened and prototypes and reference filter were removed with forceps and placed into 50 mL conical centrifuge tubes containing 40 mL of sterile, Milli-Q water. There were five different tests conducted: 1) no film (i.e. holders only, spiral versus lattice holders), 2) unpoled PVDF film (spiral versus lattice holders), 3) poled PVDF film (spiral versus lattice holders), 4) unpoled versus poled PVDF film (in spiral holder), and 5) unpoled versus poled PVDF film (in lattice holder). Each test was repeated at least three times. Dynamic blanks of lattice and spiral prototypes with unpoled or poled PVDF film (matched to test type) and reference PTFE filter were also analyzed and their values were subtracted from test results. Microbial extraction and staining/counting were conducted as described above (section 3.3.1.3) except that reference filters, all blanks, and prototypes with no film required staining of 1 mL of extraction suspension due to lower numbers of bacteria (the 1:1:8 ratios of sample extraction to formalin to Acridine Orange stain, respectively, remained

the same). For details about experimental quality control and testing of the calm air settling chamber's experimental conditions, see supplemental material.

3.3.4 Statistical Analysis

Statistical assumptions for normality and homogeneity of variance were assessed using residual plots. One-way analysis of variance (ANOVA) was performed to compare differences in extraction efficiencies across the tested materials and microorganisms and to compare bacterial collection efficiencies across the different prototype designs and film setups. ANOVA was followed by Sheffe's post-hoc test for multiple comparisons of means which is a very robust method to investigate any and all contrasts (Oehlert, 2000). Univariate general linear model (GLM) was also run to investigate complex interactions for microorganism extractions and collection efficiencies. All analyses were performed using SPSS Statistics Premium Edition, v23 (IBM Corporation, 2011) with $\alpha = 0.05$.

3.4 Results and Discussion

3.4.1 Microorganism Extraction Tests

Figure 3-3 presents the recovery efficiencies of spiked microorganisms from the test materials following an extraction procedure of vortex mixing and ultrasonic agitation. For average extraction efficiencies across tested materials, one way ANOVA revealed that there was no statistically significant effect of microorganism type ($F(2, 75) = 1.202$, $p = 0.306$). The average extraction efficiencies across all microorganism types for PVDF, nylon, acrylic, ESDs and PTFE filters were as follows: $104.62 \pm 11.78\%$, $112.55 \pm 15.48\%$, $120.13 \pm 16.07\%$, $63.80 \pm 11.80\%$ and $80.14 \pm 15.48\%$, respectively. For average extraction efficiencies across spiked microorganisms, there was a statistically significant effect of material type ($F(4, 73) = 21.629$, $p < 0.001$). Post-hoc Sheffe's test

showed that PVDF gave statistically significant better average extraction efficiency than the ESDs ($p < 0.001$) and PTFE filters ($p < 0.05$), but was neither significantly different from the 3D-printed nylon ($p = 0.855$) nor from 3D-printed acrylic ($p = 0.264$). The average extraction efficiencies of the ESDs and PTFE filters were not statistically significantly different ($p = 0.232$).

A GLM was run to investigate effects of test material type and microorganism type on spiked sample recovery. The overall corrected model was statistically significant ($F(14) = 9.391$, $p < 0.001$, $R^2 = 0.676$, adjusted $R^2 = 0.604$). Significant predictor terms included material type ($F(4) = 26.263$, $p < 0.001$), spiked microbe species ($F(2) = 3.461$, $p < 0.05$), and the interaction term between material and microbe species ($F(8) = 2.402$, $p < 0.05$). 100% extraction efficiency obtained in the present study for PVDF supports the previous findings of Therkorn et al. (2016) for extraction of 2 and 5 μm test particles when using a similar ultrasonic bath extraction procedure. To my knowledge, this study is the first to report microbial extraction efficiencies for 3D-print materials. Since ~100% extraction efficiency was obtained for both 3D-print materials tested here, this suggests that there should be minimal loss of collected particles during post-sampling analyses and collected microorganisms could be extracted from the film holders to further increase the total microbial sample quantity. Since the nylon has a relatively high heatproof temperature (80°C) compared to the acrylic (48°C), the nylon was chosen to produce the prototype samplers in all following experiments since future outdoor field tests may be done during summer.

The average extraction efficiency obtained for the PTFE filters (~80%) is similar to previously reported results. Burton et al. (2005) found a 69-87% extraction efficiency

when extracting *B. atrophaeus* spores from PTFE filters (1 μm pore size) using an extraction fluid of 0.1% (w/v) sterile peptone water with 0.01% Tween 80, 2 min vortex, and 15 min ultrasonic bath. The lowest extraction efficiencies from the ESDs (~64%) were most likely due to the fibrous nature of this material. Previous studies have reported difficulty with extraction from ESDs, particularly in the absence of Tween (Adams et al., 2015; Noss et al., 2010).

Extraction efficiencies greater than 100% obtained in the present study for PVDF and the 3D-print materials may be explained by the use of a rigorous ultrasonic bath setup, which could have broken up or fragmented microorganism agglomerates. The ultrasonic bath procedure used in the present study was optimized by adding detergent to the bath water to reduce water surface tension (Therkorn et al., 2016) and by holding the centrifuge tubes with clips to the sides of metal cages inserted into the water. The metal cages had a minimal surface area touching the bottom of the ultrasonic bath to reduce blockage of the ultrasonic pulses and the metal cages served to further reflect the ultrasonic pulses. The optimization of this setup was verified by observing the deterioration of pieces of aluminum foil in centrifuge tubes while adjusting the cage/ultrasonic bath setup (data not shown). Extraction efficiencies greater than 100% have also been previously reported in other studies when using a more mechanically rigorous extraction procedure (wrist-motion shaker agitation) (Burton et al., 2005).

3.4.2 Calm Air Settling Chamber Test Condition Measurements and Quality Control

Prior to conducting prototype tests in the calm air chamber, the experimental conditions of aerosolized *P. fluorescens* bacteria were investigated using the APS and

aerosol electrometer. Figure 3-4 presents the average aerosolized particle number concentration and net aerosol current of *P. fluorescens* bacteria during 60 min of aerosolization and 150 min of settling in the calm air settling chamber. The initial suspension concentration of the *P. fluorescens* bacteria was 1.06 x 10^9 cells/mL. This suspension was diluted by a factor of 20 to 5.3 x 10^7 cells/mL so as to not exceed the upper counting limit of the APS (~2000 particles/cm^3). Actual prototype tests were run without diluting the bacterial suspension, so Figure 3-4 has been scaled up by a factor of 20 to show estimated values for bacteria concentration and net aerosol current during actual tests (net aerosol current is proportional to particle number concentration). The number of aerosolized bacteria in the chamber rose sharply within the first 15 min, then remained relatively stable during 60 min of aerosolizing. The net aerosol current continued to increase until aerosolization was stopped; it then decreased sharply. This suggests that the most highly charged particles were lost to the chamber surfaces and/or deposited onto the samplers while particles carrying lower charge settled as expected. The number of charges per particle ranged from 25 negative to 765 positive with a mean of 214.99 ± 292.47 charges per particle. This mean is similar to the number of elementary charge units reported by Wei et al. (2014) for outdoor/indoor culturable bacterial aerosols.

The homogeneity of settled PSL particles of 5.0, 3.1 and 1.0 μm diameter is presented in Table 3-1. The observed coefficients of variation ranged from ~9 to 38% for 5.0 to 1.0 μm PSL, respectively. The increased variability for the settling 1.0 μm particles was expected since smaller particles are affected by Brownian motion and have higher electrical mobility due to low mass and a high net charge (Orr and Keng, 1976). Thus, the

rotating platform was introduced into the chamber, and the homogeneity of settling *P. fluorescens* bacteria was found to be ~9% across the platform surface. A coefficient of variation of < 10% is recommended for PM2.5 concentration measurement uncertainty (total precision) by collocated Federal Reference Method instruments according to 40 CFR Part 58; as there are no such standards for bioaerosol or settling chambers, this benchmark was applied to the present study. Therefore, the addition of the rotating platform was determined to provide sufficient sample homogeneity to proceed to prototype design testing.

3.4.3 Performance of Passive Bioaerosol Sampler Prototypes

After establishing the test conditions in the calm air settling chamber, the collection efficiencies of the two prototype designs – lattice and spiral (Figure 3-1) – when sampling a mixture of two bacteria were compared in the chamber. Table 3-2 describes the bacterial suspensions aerosolized from the Collison nebulizer for each day of testing. The average mix of *P. fluorescens* to *B. atrophaeus* bacteria throughout all test repeats was 34.79% to 65.21%, respectively. For a given day, 2-3 tests were performed and bacteria were stored in the refrigerator when not in use; there were no differences in bacterial suspension concentrations when comparing pre and post storage in the refrigerator (data not shown).

Figure 3-5 shows the collection efficiencies of the two prototypes and three tested film conditions (1. no film = holders only, 2. unpoled PVDF film, and 3. poled PVDF film). The results consist of three repeats for each combination of prototype and film. Across the three different tested film conditions, there was no statistically significant differences in collection efficiencies of the lattice and spiral prototype designs ($t(22) =$

0.04, $p = 0.97$). Therefore, the collection efficiency data of the two prototype designs were pooled; ANOVA revealed a statistically significant effect of film condition ($F(2, 27) = 14.505$, $p < 0.001$). The average relative collection efficiencies (normalized to reference filter) of the holders only (no film), unpoled PVDF film, and poled PVDF film were 0.70 ± 0.45, 2.86 ± 1.37 and 4.51 ± 1.77, respectively. Scheffe's post hoc test showed that the holders only (no film) gave significantly lower collection efficiency than both unpoled and poled PVDF films ($p < 0.05$ and $p < 0.001$). The poled PVDF film provided the greatest overall collection efficiency with statistically significantly greater collection of bacteria than the unpoled film ($p < 0.05$). A GLM showed that the prototype film condition was a statistically significant predictor term ($F(2) = 12.976$, $p < 0.001$). The corrected model was statistically significant ($F(5) = 5.262$, $p < 0.05$, $R^2 = 0.523$, adjusted $R^2 = 0.424$).

The results suggest that the prototype holders by themselves did not influence particle collection as the relative collection efficiencies were close to unity, i.e., close to that of reference PTFE filter results. Therefore, the increased collection efficiencies of the holders with PVDF films can be considered a result of that film's addition. The unpoled PVDF film provided an increased relative collection efficiency, which was 4.09 times higher than that of the control: likely due to the presence of an electrostatic charge on the film surface. Increased collection efficiency of particles of similar size to bacteria was also reported by Therkorn et al. (2016) upon addition of insulating plastic film materials to a parallel plate microchannel setup in a wind tunnel. Compared to the holder only, the integration of poled PVDF into the prototype samplers provided a relative bacterial collection efficiency of 6.44 – statistically significant increase over the unpoled film

condition. This result is likely due to the polarization of the PVDF film which has also been previously shown to increase particle collection efficiency compared to non-polarized fluorocarbon polymers. Additionally, since the PVDF film is permanently polarized until the film is heated to high temperatures (above ~100°C), the capture of charged particles is likely to be more stable and predictable compared to electret-like passive aerosol samplers (Nalwa, 1995; Therkorn et al., 2016). The particle capture performances of the spiral and lattice prototype sampler designs were not statistically significantly different; of the two, the spiral sampler design with poled PVDF film will be advanced to next stages of sampler development - outdoor field testing. This spiral design has been chosen because it required less film preparation, was easier to integrate with the film, and held the film more tightly, which is important for maintaining the proper sampler configuration (fixed air channel distance between films) in the field.

3.5 Conclusions

Here, I present prototype testing for a passive bioaerosol sampler concept using polarized ferroelectric polymer film (PVDF) to enhance electrostatic capture of bacterial aerosol mixture. Prototype testing was performed in a modified, compact calm air settling chamber to investigate how charged, settling bacteria may be diverted from their settling paths and captured by prototype sampler designs. This chapter presents the first adaptation of a calm air settling chamber to bioaerosol studies with a coefficient of variation of settling bacteria onto a rotating platform of < 10%. The testing compared two passive bioaerosol sampler designs, lattice and spiral, and three film conditions for these designs – holders only (no film), unpoled, unoriented PVDF film, and poled, oriented

PVDF film. The integration of poled PVDF film into the samplers provided a statistically significant increase of mixed bacterial aerosol collection by a factor of 6.44.

This study further refines the development of a field deployable passive bioaerosol sampler using polarized ferroelectric polymer and a nylon, 3D-printed film holder. Investigation of the extraction efficiencies of different bacterial and fungal spore types showed that 100% extraction from both the PVDF film and 3D-print material used to produce the prototypes can be expected in analyses. This presents the first results and application of 3D-printing for bioaerosol sampler prototyping and testing. Since the spiral design was more user friendly and there were no statistically significant differences in the performances of the two prototype designs, the spiral will be advanced to the next stage of passive sampler development – outdoor field testing. Overall, the presented research provides key evidence for the proof of concept in using the prototype passive sampler to significantly enhance the capture of bioaerosol particles compared to passive deposition alone (i.e., the PTFE filters). Not only does this research present the final refinements to the sampler's design prior to field testing, this research may also serve as a framework for future prototype testing of new passive bioaerosol sampler technology.

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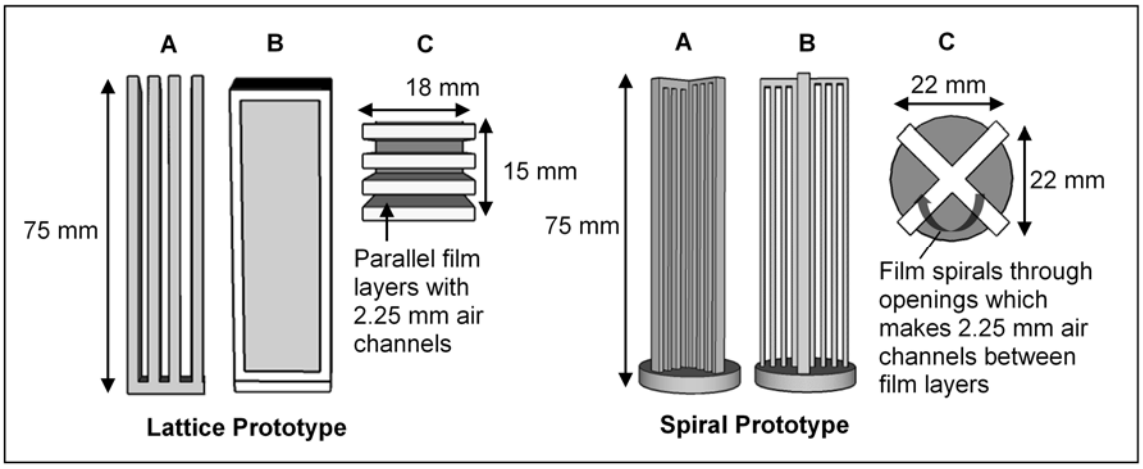

Figure 3-1.

Side (A), front (B), and top (C) views of the lattice and spiral sampler prototypes, respectively.

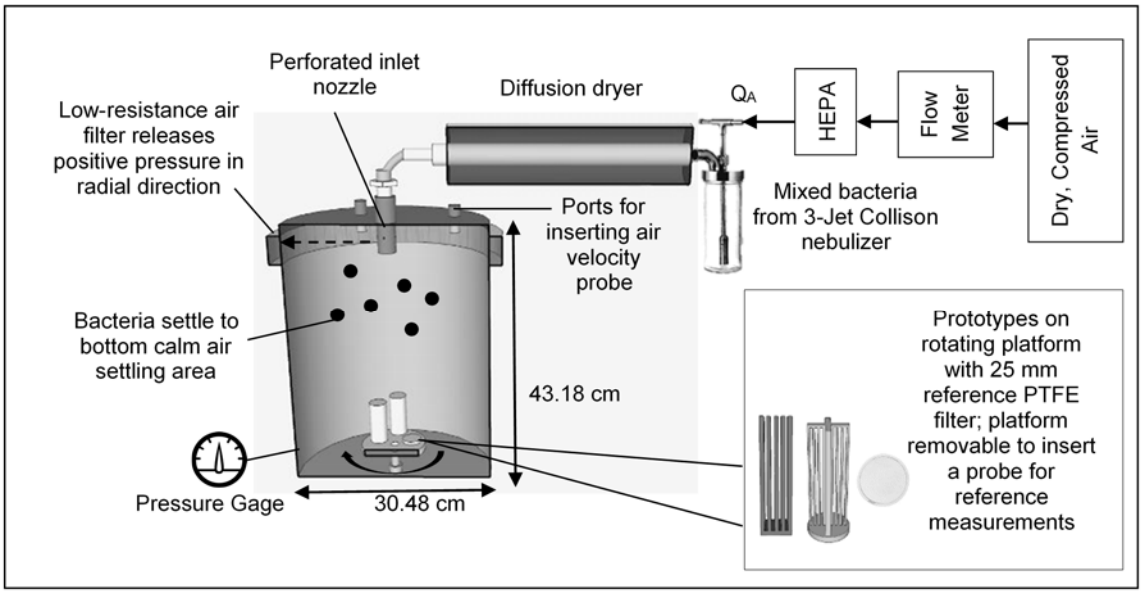

Figure 3-2.

Experimental setup for modified, compact, calm air settling chamber.

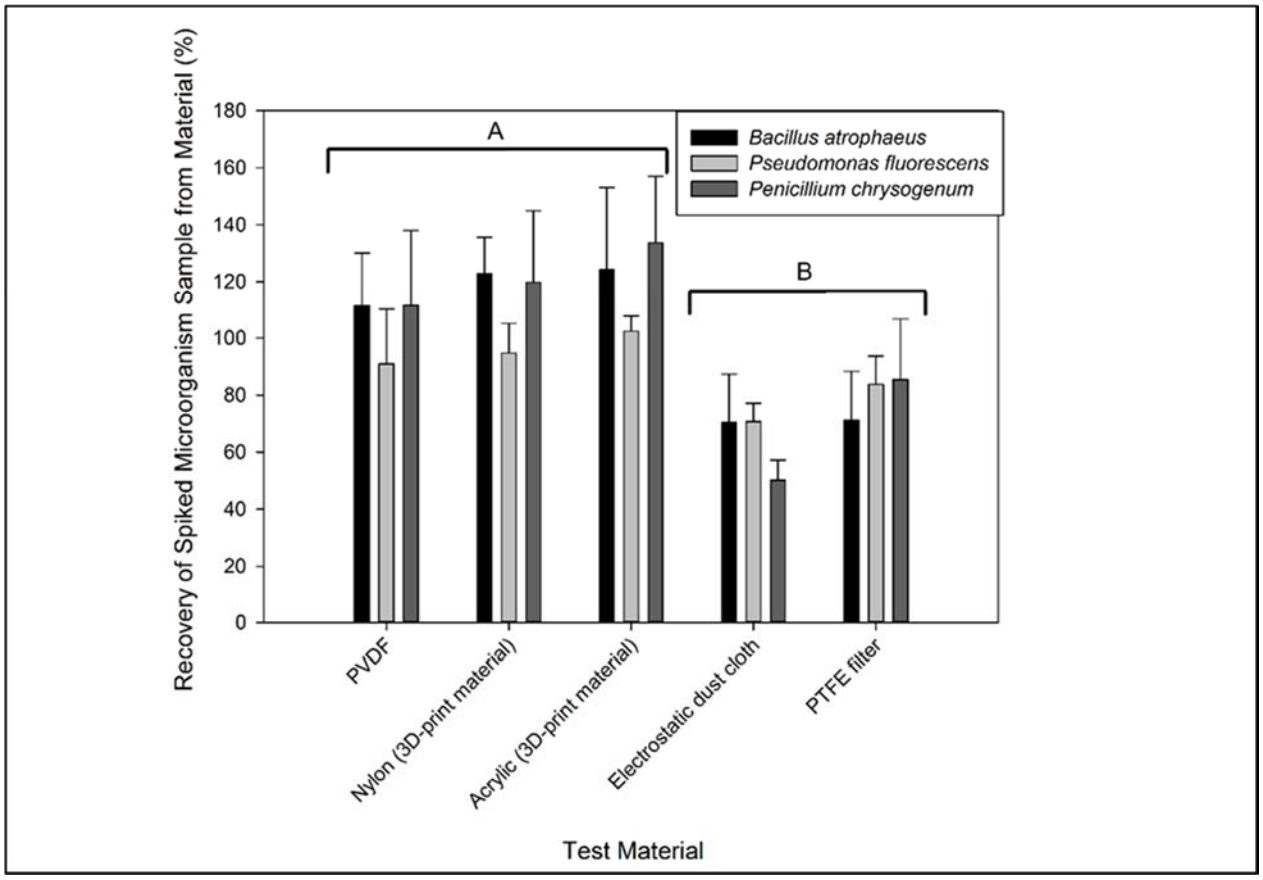

Figure 3-3.

Percent recovery of spiked microorganism samples from test materials. Groups A and B were statistically significantly different ($p < 0.05$) while the recoveries from materials within A and within B were not statistically significantly different from each other. The microbial suspensions were spiked onto the test materials to produce five repeat test material coupons per microorganism species. There were also three blanks included per test material for which sterile, Milli-Q water was spiked onto the material surface in the same manner.

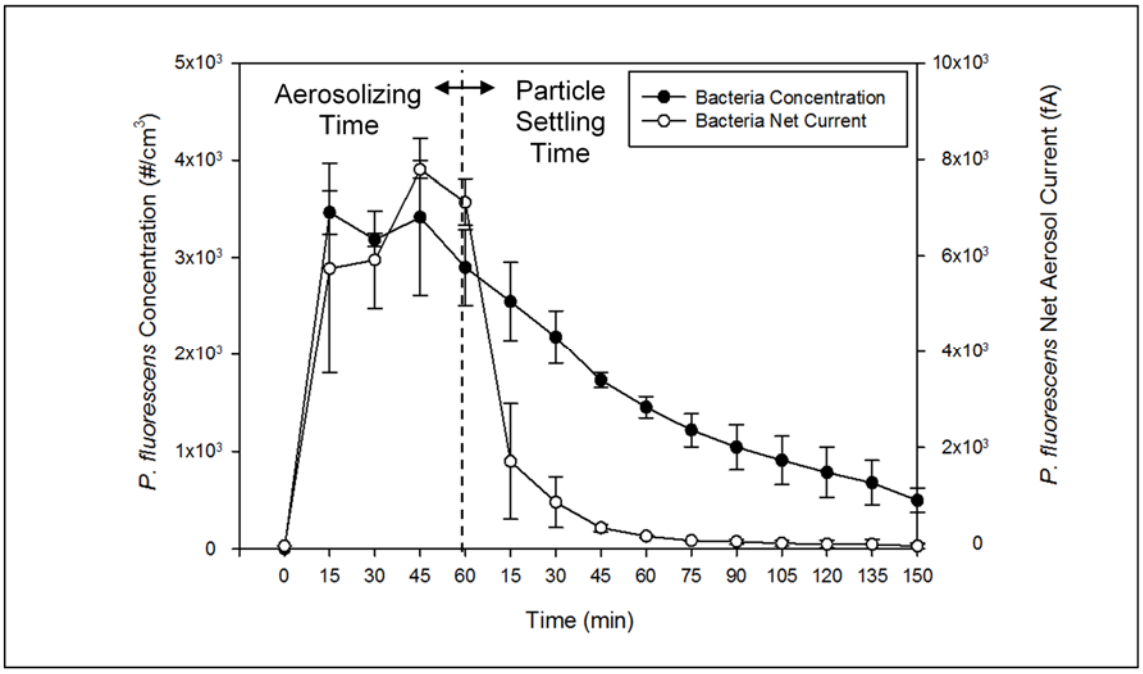

Figure 3-4.

Average particle number concentration and net aerosol current of *P. fluorescens* bacteria aerosolized into the calm air settling chamber with a bacterial suspension of 5.3 x 10^7 cells/mL. Chamber condition measurements included particle number size distribution measured by an Aerodynamic Particle Sizer Spectrometer (APS, model 3321, TSI Inc.) and aerosol net charge measured by an Aerosol Electrometer (model 3068, TSI Inc.). Three repeat experiments were conducted to investigate particle number concentrations that could be expected in the chamber during experiments. For these repeat experiments, twenty second sample measurements were taken from the chamber every 15 min during aerosolization (60 min total) and up to 150 min during settling time. The three test runs were averaged together to estimate the average aerosolized bacteria number concentration and net aerosol charge (current in electrometer) in the chamber during prototype testing.

Table 3-1.

Calm Air Chamber Particle Homogeneity	
Coefficient of variation (%) for aerosol homogeneity (without rotating platform)	5.0 µm PSL: 9.00 ± 6.96% 3.1 µm PSL: 15.02 ± 5.04% 1.0 µm PSL: 37.77 ± 18.29%
Coefficient of variation (%) for aerosol homogeneity (with rotating platform)	*P. fluorescens* bacteria (0.8 µm aerodynamic diameter): 8.83 ± 7.09%

Table 3-2.

Bacterial Suspension Conditions during Prototype Testing in Calm Air Chamber			
Test Day	***P. fluorescens* (%)**	***B. atrophaeus* (%)**	**Initial Aerosolized Suspension Concentration (cells/mL)**
1	36.99	63.01	2.70×10^9
2	40.86	59.14	3.14×10^9
3	29.01	70.99	3.23×10^9
4	31.70	68.30	2.92×10^9
5	48.32	51.68	4.07×10^9
6	21.86	78.14	2.70×10^9

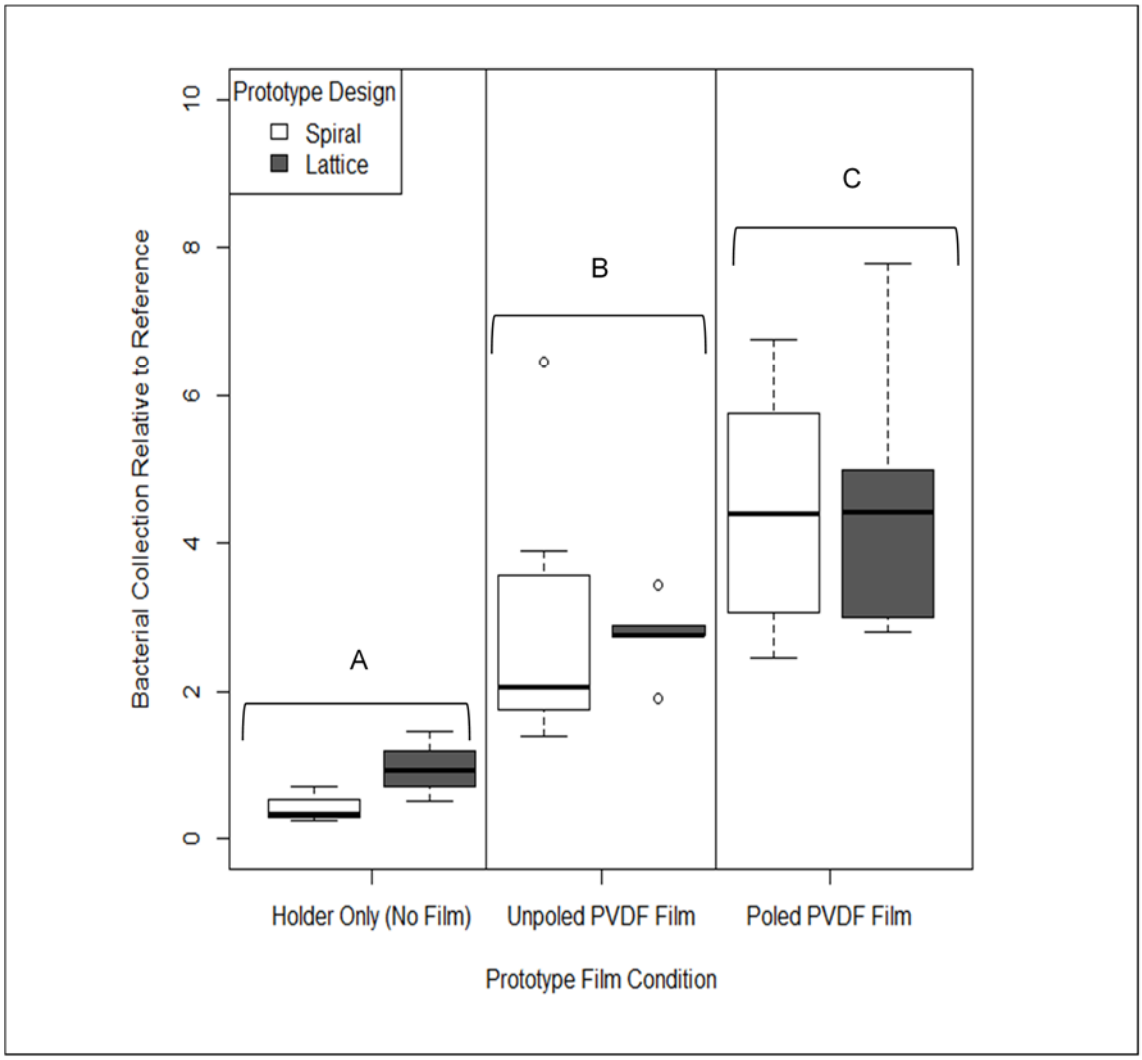

Figure 3-5.

Bacterial collection by different prototype designs (spiral vs. lattice) and film conditions (no film, unpoled film and poled film) in the calm air chamber relative to reference 25 mm PTFE filters. Groups A, B and C are statistically significantly different from each other while results within each group are not significantly different from each other. The results consist of three repeats for each combination of prototype and film.

CHAPTER 4. OUTDOOR FIELD TESTING OF A NOVEL PASSIVE BIOAEROSOL SAMPLER USING POLARIZED FERROELECTRIC POLYMER FILMS

Material in this chapter has been previously submitted for publication as:

Therkorn, J.; Thomas, N.; Scheinbeim J.; Mainelis, G. Outdoor Field Testing of a Novel, Passive Bioaerosol Sampler using Polarized, Ferroelectric Polymer Films. *Journal of Exposure Science and Environmental Epidemiology* **2016**, submitted.

4.1 Abstract

A new, passive bioaerosol sampler can improve exposure science and environmental epidemiology by allowing for easier, more cost-effective sampling over wider spatiotemporal scales. Here, I present the first outdoor field testing of a novel, passive bioaerosol sampler developed using a polarized, ferroelectric polymer ((poly)vinylidene fluoride, PVDF): the Rutgers Electrostatic Passive Sampler (REPS). Four 10 day long field campaigns were conducted in highly varied meteorological conditions to compare collection efficiencies of REPS to active reference (Button Aerosol Samplers) and passive sampler controls (PTFE settling filters and agar settling plates). Compared to passive PTFE filters, REPS enhanced passive deposition of total microorganisms by ~7-fold. REPS also significantly enhanced passive capture of culturable bacteria and fungi by 82% and 77%, respectively, and collected 65% of the culturable bacteria that the active Button Samplers collected. Since the Buttons operated at 4 L/min, REPS had an average equivalent sampling rate of 2.6 L/min and 1.0 L/min for culturable bacteria and total bacteria + fungi, respectively – about an order magnitude greater than that of the PTFE filters. These results suggest that REPS passively collects

microorganisms comparably to an active sampler over long sampling durations, especially for culturable bacteria due to better preservation of culturability.

4.2 Introduction

Bioaerosols are airborne particles of biological origin, including viable and nonviable microorganisms, pollen grains, and fragments, toxins, and particulates shed or produced by living organisms. Exposure to bioaerosol particles is of concern as it can potentially cause negative health effects. For example, infectious diseases, like influenza, can be spread by dispersal of microorganisms through coughing and sneezing; acute toxic effects can result from inhalation of fungal spores or organic dusts, while health effects like asthma, allergies, and cancers can result from chronic inhalation of allergens and mold toxins (Cox and Wathes, 1995; Douwes et al., 2003; Linaker and Smedley, 2002). Example environments that are of particular concern for exposure to high concentrations of bioaerosol particles and/or pathogens of concern include healthcare facilities, agricultural animal houses and residences with moisture problems post flooding (Cox and Wathes, 1995). Additionally, widespread airborne dispersal of pathogens through bioterrorism has been called the most under-addressed threat related to terrorism (United Nations, 2006).

At the same time, environmental bioaerosols constitute a substantial fraction (about 5 to 35% on average) of all airborne particulate matter and can be found throughout the atmosphere (Bauer et al., 2008; Elbert et al., 2007; Fröhlich-Nowoisky et al., 2009; Held et al., 2008; Hock et al., 2008; Jaenicke, 2005; Kellogg and Griffin, 2006; Smith et al., 2010; Womiloju et al., 2003). For example, about 20% of all flowering plants depend upon wind pollination (Ackerman, 2000). The widespread presence of bioaerosols and their environmental health impacts call for sampling technology that can

provide exposure data over relevant spatiotemporal scales. However, there is a lack of sampling technology available to meet the needs for bioaerosol exposure assessment, especially over longer time scales (Peccia et al., 2008). Furthermore, confounding factors, like the presence of diverse bioaerosol agents with varying levels of toxicity, have contributed to a lack of understanding of bioaerosol exposure-response relationships; in turn, this has also contributed to the non-existence of health-based occupational exposure limits for bioaerosol (Eduard et al., 2012).

One reason for the difficultly in conducting bioaerosol sampling is the extensive personnel time and resources required to conduct long term trend studies and personal sampling for bioaerosols (Cartwright et al., 2009). Typically, bioaerosol sampling is done with active, pump-based samplers which require noisy, heavy pumps, and external power to these pumps (Reponen et al., 2011). Personal pumps are also available, but they can be cumbersome, provide limited flowrates, and the wearing of sampling pumps may affect exposure estimates by causing the wearer to modify their typical behaviors (Cherrie et al., 1994; Wood, 1977). As a result, reliance on active sampling limits when and where bioaerosol sampling can be performed, and it affects the quality of the sample obtained. Furthermore, active sampling desiccates/damages collected microbes, and necessitates frequent sampler media replacement (Cartwright et al., 2009; Jensen and Schafer, 2003; Stewart et al., 1995; Zhen et al., 2013). This negatively impacts capabilities to conduct bioaerosol exposure assessment for applications like epidemiological research where many cost-effective replicate samples are desired (Adams et al., 2015).

Alternatively, a passive bioaerosol sampler, where no pumps or power sources are required, could reduce the logistical burdens and financial costs of active sampling and

allow for long term sampling in any location indoors or outdoors. In order for passive sampling to be a practical solution for exposure assessment, it has to be able to detect the biological agents of concern while maintaining simplicity in its use (Tovey et al., 2003). A new type of passive bioaerosol sampler, herein termed the Rutgers Electrostatic Passive Sampler (REPS), has been developed using a ferroelectric polymer film ((poly)vinylidene fluoride, or PVDF) which can be permanently polarized for typical environmental sampling applications(Therkorn et al., 2016a; Therkorn et al., 2016b). This new passive, bioaerosol sampler is designed to hold parallel layers of PVDF film with thin air channels between the film layers to significantly enhance passive, electrostatic capture of airborne biological particles (Therkorn et al., 2016a; Therkorn et al., 2016b). By using this sampler design, PVDF film has also been shown to collect particles representing the full size spectrum of typical bioaerosol particles (nano to micron-size) (Therkorn et al., 2016a). A spiral shaped design of the sampler was shown to provide simple, user-friendly sampling procedures with ~100% extraction of collected microorganisms off of the sampler for analyses (Therkorn et al., 2016b).

Here, I present the results of the first outdoor field testing of REPS. Outdoor field testing was performed using 10 day long campaigns in highly varied weather conditions. The goals of these field campaigns were to compare collection efficiencies of REPS versus active reference samplers and passive sampler controls for total and culturable bacteria and fungi. This research is the first to investigate the application of passive bioaerosol sampling for long term outdoor field sampling as a method to study bioaerosol exposure trends. This research is also the third phase of a larger project aimed at

delivering and testing a field-deployable, passive bioaerosol sampler as a novel exposure assessment tool.

4.3 Materials and Methods

4.3.1 Sampling Site

Figure 4-1 presents the setup of the outdoor sampling site. Four 10 day long outdoor field sampling campaigns were conducted in an organic garden on an agricultural college campus in New Jersey. The field campaigns were conducted between September and December, 2016. Samplers were placed on a table about 1 m above the ground inside a protective enclosure. The top of the enclosure was covered with a tarp to protect samplers from large falling debris and did not impede air movement through the enclosure.

The tested samplers included three types of passive samplers, one type of continuous active, reference control, and two types of real-time aerosol monitoring instruments. For each sampling day, the passive samplers were: 1) six spiral REPS with polarized (poly)vinylidene fluoride (PVDF) film (Therkorn et al. 2016b) (Figure 4-2),2) six passive PTFE filters (2 μm pore size, 25 mm diameter, SKC Inc.), and 3) six agar settling plates (90 mm, Fisher Scientific, Waltham, MA). The active, reference controls were three Button Aerosol Samplers (SKC Inc., Eighty Four, PA) operated at 4 L/min with the same PTFE filters as the passive PTFE filters. The real-time, aerosol monitoring instruments were 1) an Aerotrak Handheld Optical Particle Counter (OPC, model 9306, TSI Inc., Shoreview, MN), and 2) a DustTrak DRX Aerosol Monitor (DRX, model 8534, TSI Inc.). The preparation steps, operation and analysis procedures for these samplers are detailed below.

A portable weather station (WS-2080 Wireless Weather Station, Ambient Weather, Chandler, AZ) was also included with the sampling site to continuously record the temperature, relative humidity, wind speed and peak wind gust in five minute intervals (Figure 4-1B). There was precipitation for 12.5% (5/40) of all sampling days; on these days, small, hard plastic rain covers were placed over all samplers to protect from direct exposure to rain. Openings were cut into the covers to allow free airflow.

4.3.2 Active Reference Samplers: Button Aerosol Samplers

Three Button Aerosol Samplers operating side by side served as the active reference samplers for field campaigns (Figure 4-1C). The results obtained from the Button Samplers were averaged each day to estimate variability in the active, reference sampler results. Each 24 hour sampling period started with three clean Button Samplers and three fully recharged, recalibrated pumps (AirChek XR5000 Sample Pump, SKC Inc.). To clean the Button Samplers, they were autoclaved, soaked overnight in ethanol, and then wiped dry with Kim Wipes (Kimberly-Clark Professional, Roswell, GA) to remove any residue. Immediately prior to field deployment, fresh PTFE filters were loaded into each Button Sampler in a laminar flow hood (NuAire Class II, Type A2, Plymouth, MN) using autoclaved forceps, and the Button Samplers were transported to/from the field in a clean, unused plastic zip bag.

After returning each set of Button Samplers to the lab every 24 hours, the filters were removed with autoclaved forceps and each filter was placed into a separate 50 mL conical centrifuge tube (Falcon Tubes, Corning Inc., Tewksbury, MA) containing 5 mL of autoclaved Milli-Q water (EMD Millipore Corp., Milli-Q Direct 8, Billerica, MA). These tubes are pre-sterilized during manufacture. The particle extraction, staining with

Acridine Orange to count total bacteria and fungi, and counting procedures were the same as described by Therkorn et al. (2016b) with the addition of one step: excess stain from the black polycarbonate filter was removed by filtering 1 mL of 70% ethanol immediately prior to slide mounting. Briefly, particles were extracted from filters using a 2 minute vortex and 10 minute ultrasonic agitation in sterile, Milli-Q water. Then, a 100 µL aliquot of extraction suspension was used for Acridine Orange staining of bacteria and fungi, and these microbes were then counted by epifluorescence microscopy. The added ethanol washing step helped to remove non-specific staining and reduces the brightness of the background (An and Friedman, 2000).

For each campaign day, the Button Sampler filter with the highest number of stained bacteria and fungi was used to prepare spread plates to estimate the culturable fraction of bacteria and fungi. Spread plates were made in triplicate for culturable bacteria (three plates with tryptic soy agar (TSA, Becton, Dickinson and Co., Sparks, MD) with 200 mg cycloheximide antifungal agent (Sigma-Aldrich Co., St. Louis, MO) per liter agar) and culturable fungi (three plates with malt extract agar (MEA, Becton, Dickinson and Co.)). Spread plates were made by evenly spreading 100 µL aliquots of the particle extraction suspension onto each plate. All plates were incubated at room temperature for five days and new colony forming units (CFU) were counted every 24 hours. Since the extraction suspension for each filter was 5 mL, it was divided into parts for staining and culturable analyses, and the entire extraction suspension did not have to be used: 100 µL was used for staining/counting, and for the filter with highest total bacteria + fungi, 100 µL was used per spread plate (two types of agar plates, three plate repeats = 600 µL total).

4.3.3 Passive Samplers

The passive PTFE filters and REPS were placed onto 3D-printed pedestals 2 cm high to allow for free air movement around the samplers (Figure 4-1D). The pedestal onto which each passive sampler was placed was a randomized order for each campaign. Each passive sampler was secured to its pedestal base with a small metal clip. The pedestals were cleaned by wiping them with 70% ethanol before each new campaign. Prior to deployment, the PTFE filters were autoclaved and the PVDF films were cleaned and assembled into the 3D printed film holders as described elsewhere (Therkorn et al., 2016b). Briefly, the PVDF films were wiped with 70% ethanol and rinsed with sterile, Milli-Q water. The 3D printed film holders were cleaned before each campaign by scrubbing with a bristle brush in 10% Alconox detergent solution (Alconox Inc., White Plains, NY), then sonicating in sterile, Milli-Q water for eight hours to ensure all contaminants on the surface and in the interstitial spaces of the 3D printed film holders were removed. The PTFE filters and REPS were transported to/from the field in individual 50 mL conical centrifuge tubes.

The PTFE filters and REPS were deployed for the entirety of a given sampling campaign; after a sampling campaign was finished, particles were extracted from these samplers immediately and suspensions were subdivided for staining and culture analyses. Total bacteria and fungi were stained and counted as described above (section 4.3.2) except the REPS sampler's extraction suspensions were 40 mL of sterile Milli-Q water to fully submerge the samplers in the centrifuge tubes (Figure 4-2C). Thus, due to this extra dilution of REPS' microbial samples, 1 mL of extraction suspension was used for staining/counting. The passive PTFE filters used an extraction volume of 5 mL (same as

for the filters from the Button Aerosol Samplers), but an aliquot of 1 mL was also used to stain/count microbes from the passive PTFE filters due to the expectation that there would be lower total yield numbers on average. Spread plates were also made, incubated and counted as described above (section 4.3.2) using the two PTFE filters and the two REPS which showed the highest total numbers of stained bacteria and fungi per campaign. The spread plates for the passive PTFE filters and REPS had to maintain the use of 100 µL aliquots as the use of higher volumes results in too much liquid on the plates. In summary, each REPS' extraction volume was 40 mL, and it was divided as follows: 1 mL for staining/counting, and for the two REPS with highest number of stained bacteria + fungi, 600 µL total was taken from each REPS' extraction suspension for spread plates. For each of the passive PTFE filters, the extraction volume was 5 mL, and it was divided as follows: 1 mL for staining/counting, and for the two PTFE filters with the highest number of stained bacteria + fungi, 600 µL total was taken from each filter's extraction suspension for spread plates.

The third type of passive sampler was the agar settling plates. Three TSA plates and three MEA settling plates were deployed each sampling day between 0800 and 1200 hours for a four hour sampling period. Four hours is the maximum recommended amount of time for which settling plates can be used without significantly affecting microbial growth through agar desiccation (Baird et al., 2000; Sandle, 2015). After the four hour exposure, settling plates were closed, returned to the lab, and incubated at room temperature for five days. New CFU's were counted every 24 hours. The total number of CFU's for the bacteria and fungi were determined at the end of each campaign as the sum of CFUs from all sampling days multiplied by a factor of six because they were deployed

for 4 out of 24 hours daily; the assumption in doing so is that the four hour period is representative of 24 hours – an inherent limitation in using settling plates for long term bioaerosol sampling. For ~3% of all settling plates (7/240), the number of CFU's were too high to count the entire surface of the settling plates; so, the plates on these days were divided into four quadrants, one quadrant was counted and the CFU multiplied by four for that day.

4.3.4 Real-Time Aerosol Monitoring Instruments

The DRX and OPC (Figure 4-1A) were run for 60 min each sampling day starting between 0800 and 1200. The OPC monitored particle number concentrations in the size range of 0.3 to 10 μm. This data was used to estimate the daily biological fraction of particulate matter by dividing the average total number of stained bacteria and fungi obtained from daily Button Samplers by the average total number of particles > 1 μm registered by the OPC across a given sampling campaign. Single and agglomerated bacterial and fungal particles are typically expected to be in the size range of particulate matter from 1 to 10 μm (Gorny et al., 1999; Reponen et al., 2011; Yamamoto et al., 2014). Since the OPC was only run for 60 min each sampling day, the daily OPC values were averaged across each campaign to reduce variability in calculated daily biological fractions.

The DRX monitored particle mass concentrations for PM10 and PM2.5. These data were compared to a nearby New Jersey PM2.5 sampling station (NJDEP Air Quality Monitoring Station at Rutgers University, New Brunswick, NJ) which provides continuous 1 hour averages for PM2.5 using beta attenuation monitoring (BAM-1020 Continuous Particulate Monitor, Met One Instruments Inc., Grants Pass, OR). The

NJDEP station is located about 1.10 km from the sampling site and therefore served as reference to investigate local particulate pollutant levels at the sampling site in relation to the nearby station. The DRX is factory calibrated with the respirable fraction of standard ISO 12103-1, A1 test dust. Thus, assumptions are made about the relationship between light scattering of the measured particles and their mass concentration. The DRX was therefore calibrated for use at the sampling site against two gravimetric PM2.5 Personal Modular Impactors (PMI, SKC Inc., Eighty Four, PA). A correction factor of (-)12% was applied to the DRX measurements for PM2.5 (and for PM10 which was of similar mass concentration to the PM2.5 across all campaigns). For more details on the DRX calibration, see the supplementary information.

4.3.5 Blanks

For each campaign, one PTFE filter and one REPS were used as dynamic blank controls: they were placed onto a sampling pedestal, and then removed and taken to the lab for analysis. Dynamic blanks were also taken for the Button Samplers on three randomly chosen days (three blanks total per campaign) by assembling extra Button Samplers, transporting to the field and onto the sampling site table, and then immediately returning the sampler in its transport bag. Blanks for agar settling plates were randomly chosen plates representing 20% of the total number of agar plates prepared. Blanks for agar spread plates were prepared from each of the blank samplers. Mean blank values were subtracted from results. Blank values for total stained bacteria and fungi were ≤ 10% of the post-sampling values for all samplers. No contamination was detected for settling plates or spread plates.

4.3.6 Statistical Analyses

Residual plots evaluated statistical model assumptions, including assumptions for normality and homogeneity of variance. Some of the datasets had unequal residual variances. Therefore, independent samples t-tests for equality of means assuming two-tailed significance (equal variances not assumed) were conducted to compare the collection efficiencies of REPS versus PTFE filters across campaigns. Collection efficiencies of passive samplers were determined relative to the average total bacteria and fungi collected by the Button Samplers. To compare the performances of REPS and the PTFE settling filters to agar settling plates, Welch's one-way analysis of variance (ANOVA) followed by Games-Howell post-hoc test was used to compare differences in average total bacteria and fungi CFU/cm^2 of deposition surface (projected surface area was used for REPS). To assess the relationships between passive and active sampler performances, Spearman's rank correlation coefficients were determined for the average total and culturable numbers of bacteria and fungi collected by REPS and the PTFE filters versus the active reference Button Samplers. Average equivalent sampling rates (mL/min) were determined for REPS and the PTFE filters by estimating the passive sampler collection efficiencies relative to the Button Aerosol Samplers and multiplying this value by the Button Sampler flow rate (4,000 mL/min). All analyses were performed using SPSS Statistics Premium Edition, v23 (IBM Corporation, 2011) with $\alpha = 0.05$.

4.4 Results

4.4.1 Sampling Site Conditions

Table 4-1 summarizes the meteorological conditions throughout the four field sampling campaigns. Mean temperature ranged from 19.72 ± 4.37°C for the first campaign conducted in September to 9.32 ± 5.23°C for the final campaign conducted in

December. The mean relative humidities, wind speeds and peak wind gusts were similar across the four campaigns with combined campaign averages of 68.58 ± 21.39%, 0.54 ± 0.67 m/s and 1.07 ± 1.13 m/s, respectively. Throughout each campaign, the daily relative humidity broadly ranged from about 20 to 99%. The total precipitation was different for each campaign with totals of 0, 16.76, 21.34 and 38.54 mm for campaigns 1 to 4, respectively. Figure C-1 suggests that there were local sources present at the sampling site location as the PM2.5 and PM10 mass concentrations were higher than that reported by the nearby NJDEP sampling station.

Figure 4-3 presents bioaerosol fractions of particulate matter (bacteria and fungi combined) and culturable fractions of these bacteria and fungi. Across the four campaigns, the mean percentages of biological particulate matter and percentages of these bacteria + fungi that were culturable were 5.21 ± 2.47%, 10.27 ± 4.95%, 9.71 ± 6.52%, and 3.15 ± 1.88%, and 0.36 ± 0.37%, 0.96 ± 1.35%, 0.79 ± 0.54%, and 1.11 ± 1.10%, respectively. The normalized particle number concentrations by particle size bins of the Aerotrak OPC used to estimate total number of particles greater than 1 μm are depicted in Figure C-2.

4.4.2 Sampler Performance Comparisons

For combined sampler performances across the four campaigns, there was no statistically significant effect of campaign for any given sampler metric. Therefore, to increase statistical power, all results described below are pooled sampler data across all four campaigns. Figure 4-4 presents the collection efficiencies (mean ± 1SD) of REPS and PTFE filters relative to the Button Aerosol Samplers for total bacteria and fungi across campaigns. The mean relative collection efficiency of REPS (28.93 ± 21.26%)

was statistically significantly greater than that of the PTFE filters (3.97 ± 2.10%, $t(23.45) = -5.72$, $p < 0.001$).

Figure 4-5 demonstrates the collection efficiencies of REPS versus the PTFE filters for culturable bacteria and fungi relative to the Button Aerosol Samplers for each sampling campaign. REPS had a mean relative collection efficiency for culturable fungi and bacteria of 23.98 ± 12.97% and 64.85 ± 40.57%, respectively, while the PTFE filters had collection efficiencies of 4.78 ± 2.17% and 11.07 ± 16.98% for the same culturable microbes. This culturable collection efficiency of REPS was statistically significantly greater than the mean collection efficiency of the PTFE filters for both fungi ($t(24.29) = -7.16$, $p < 0.001$) and bacteria ($t(30.82) = -5.99$, $p < 0.001$).

Figure 4-6 shows the number of culturable microorganisms across the three passive sampling methods - REPS, PTFE filters and settling agar plates – normalized to each sampler's surface area: average total CFU/cm^2 (mean ± 1SD). The mean values for average total CFU/cm^2 for REPS, PTFE filters, and settling plates for culturable bacteria were 2204.17 ± 1720.08, 302.50 ± 425.48, and 16.69 ± 8.30, respectively; for fungi, these values were 1308.33 ± 1098.98, 212.50 ± 139.76, and 22.13 ± 17.75, respectively. Compared to deposition by gravitational settling alone as used by the PTFE filters and settling plates, REPS provided statistically significantly enhanced passive deposition of culturable bacteria (Welch's $F(2, 30.67) = 24.29$, $p < 0.001$) and fungi (Welch's $F(2, 30.77) = 37.80$, $p < 0.001$). Post-hoc Games Howell multiple comparisons of means showed that the mean collection efficiencies of all three passive samplers were statistically significantly different for both culturable bacteria and fungi ($p < 0.001$ for all

comparisons, except p = 0.009 for PTFE filters versus agar settling plates for culturable bacteria collection).

Across all sampling campaigns, Figure 4-7 demonstrates equivalent sampling flow rates (mean ± 1SD) for REPS and PTFE filters. The mean equivalent sampling rates for total bacteria + fungi and culturable fungi were similar for a given passive sampler. For REPS, the mean sampling rate for total bacteria + fungi and culturable fungi was 1030.75 ± 676.66 mL/min; for PTFE filters, the mean sampling rate was 174.97 ± 86.14 mL/min. Both samplers, however, had a much better sampling performance for culturable bacteria. For culturable bacteria, REPS provided a mean equivalent sampling rate of 2593.99 ± 713.99 mL/min while the PTFE filters had a mean equivalent sampling rate of 442.68 ± 128.45 mL/min.

Figure C-6 demonstrates relationships between the average total and culturable microorganism numbers collected by the Button Aerosol Samplers and the passive samplers. Each relationship has four data points and more sampling campaigns are needed to estimate correlation coefficients between active and passive sampler performances; however, based on the current data, moderate positive (Spearman's rho, r: REPS and PTFE filters, 0.40) and strong (r: REPS, 0.80; PTFE filters, 1.00) correlations can be seen between passive sampler and Button Aerosol Sampler performances for culturable bacteria and fungi collection, respectively. For the collection of total bacteria and fungi, there was no correlation between PTFE filter performance and the Button Aerosol Samplers. REPS, however, appears to have a moderate negative correlation with the Button Aerosol Sampler's collection of total bacteria and fungi (r: -0.40).

4.5 Discussion

4.5.1 Sampling Site Conditions

With temperatures ranging from -2.28 to 32.50°C and relative humidities between 17 to 99%, there were highly varied weather conditions during the field sampling campaigns. The site was located in an active, community garden and the particulate matter pollutant levels were high relative to the NJDEP sampling station (up to 90.64 $\mu g/m^3$ for PM10 and up to 73.92 $\mu g/m^3$ for PM2.5 at the test site). The presence of local particulate pollutant sources most likely resulted from high levels of activity observed at the site, like composting, gardening, construction and vehicles passing on an unpaved road. The results suggest that most particulate pollution at the site was in the fine fraction of particulate matter as the mass concentrations of PM2.5 and PM10 were similar across all campaigns.

While the particulate pollutant concentrations were high, the measured biological fractions of particulate matter at the site (7.13 ± 5.23%) and culturable fractions of bacteria and fungi (0.82 ± 0.96%) were similar to values previously reported in the literature (Boreson et al., 2004; Glikson et al., 1995; Jaenicke, 2005; Tham and Zuraimi, 2005; Ting et al., 2010; Womiloju et al., 2003). According to Button Sampler data, the average total bioaerosol concentration for the field campaigns (bacteria + fungi) was $5.20x10^4 \pm 3.05x10^4/m^3$ with all data in the range of 10^4 to $10^5/m^3$. These values are typical for outdoor bioaerosol concentrations (Cox and Wathes, 1995). Together, these data support the broad applicability and relevance of the presented research to different sampling environments and conditions that are not specific to the sampling site chosen for this study.

4.5.2 Sampler Performance Comparisons

As compared to the performances of the PTFE filters and agar settling plates which rely on gravitational settling of particles, REPS provided significantly enhanced passive collection of total bacteria and fungi. This result was consistent across all four sampling campaigns despite highly variable meteorological conditions across the campaigns. Since the REPS samplers and the PTFE filters have similar projected diameters (22 and 25 mm), the enhanced biological particle collection of REPS reflects the optimized electrostatic capture of microorganisms between the parallel layers of polarized PVDF film (Therkorn et al., 2016a; Therkorn et al., 2016b). The especially enhanced performance of REPS for culturable bacteria collection is likely a result of both increased total collection and better preservation of bacteria culturability. The Button Aerosol Samplers desiccate the captured bacteria as the required flow rate of the active sampler continuously pulls air through the filters onto which the microorganisms are deposited. Desiccation and sampling stress on bacteria have been commonly reported in previous bioaerosol studies (Jensen et al., 1992; Li, 1999; Stewart et al., 1995; Zhen et al., 2013), and bacteria may generally be more sensitive to sampling stress than fungi (Chen and Li, 2005; Wang et al., 2001).

In order to use REPS as a quantitative exposure assessment tool, an equivalent sampling rate was estimated. With an equivalent sampling rate of ~1,000 mL/min for total bacteria and fungi and ~2,600 mL/min for culturable bacteria, REPS may passively collect microorganisms comparably to an active sampler for long duration bioaerosol sampling campaigns. For example, the choice of reference sampler for this study, the Button Aerosol Sampler, collects the inhalable fraction of particulate matter (Aizenberg et al., 2000) at 4,000 mL/min and was operated in 24 hour intervals. The Button Aerosol

Sampler has been previously used to investigate concentrations of and exposure to outdoor aeroallergens, bacteria, fungal spores, and endotoxin levels in various environments (Adhikari et al., 2003; Adhikari et al., 2004; Aizenberg et al., 2000; Kilburg-Basnyat et al., 2015; Lee et al., 2006; Toivola et al., 2002). Similarly, the IOM Inhalable Sampler and the Personal Environmental Monitor (PEM) Sampler (both SKC Inc.), are designed to sample the inhalable fraction of particulate matter and PM10 or PM2.5, respectively, and operate with air pumps at 2,000 mL/min. The IOM Sampler has been used for sampling airborne fungi in occupational settings (Green et al., 2005) and near waste composting facilities (Taha et al., 2006) while the PEM sampler has been used to investigate ambient and indoor biological content of particulate matter (Menetrez et al., 2009) and microbial species diversity (Hoisington et al., 2014). A critical difference between REPS and these active aerosol samplers is that REPS does not need an air pump which is highly beneficial because pumps have to be repeatedly recharged or plugged in. In this sampling campaign, it was operated for 10 days continuously. Future studies will investigate its usability in shorter and longer campaigns of several weeks or even months long.

Previous studies have also investigated equivalent sampling rates of other types of passive aerosol samplers. The Personal Aeroallergen Sampler (PAAS) has been compared to a two-stage cyclone sampler and found to have an equivalent sampling rate about two orders of magnitude less than that of REPS: 32 ± 6 mL/min to 66 ± 44 mL/min when sampling fungal species > 5 μm in aerodynamic diameter (Yamamoto et al., 2011). The equivalent sampling rate of the electret sampler (Brown et al., 1996; Brown et al., 1995; Brown et al., 1994) has been calibrated to be 22.5 mL/min when testing its

performance in the laboratory versus the United Kingdom's conventional Methods for the Determination of Hazardous Substances (MDHS) guidance 39/4 membrane filter pump sampling method using asbestos fibers as the test dust (Burdett and Revell, 1999).

The electret sampler is similar in principle to REPS as it also relies on electrostatic capture of particles; however, electrets are not permanently polarized as charge injection is used to obtain a remanent polarization (Therkorn et al., 2016a) and their charge steadily dissipates throughout use(Burdett and Bard, 2007). Since the electret calibration experiments were only conducted for up to 180 min, the difference in estimated equivalent sampling rates between the electret and REPS is most likely not a result of the electret losing substantial charge as would be expected during longer sampling campaigns (Burdett and Bard, 2007). The diameter of the electret (25 mm) is similar to the projected diameter of REPS (22 mm), but the total collection surface area of REPS is greater: ~ 30,765 mm^2 including film and 3D print holder versus 491 mm^2 of the electret. As suggested by Burdett and Revell (1999), the total collection of the electret sampler could have been increased by increasing the total surface area of the electret, but this was not practical given their chosen methods for preparation and analysis of the corona-poled polypropylene disc. Here, REPS offers an alternative to using a one-dimensional flat sampling disc. By coiling PVDF film into a 3D-printed film holder, the total sampling collection surface area is increased, but the entire sampler fits into a 50 mL conical centrifuge tube for practical sampler transport and removal of collected particles for analyses. In addition, use of water as extraction medium allows sample analysis by multiple methods.

The relationships between REPS and the active reference Button Aerosol Sampler's performances as depicted in Figure C-3 indicate that the high levels of particulate matter pollution may have caused REPS to reach a saturation point during testing. This saturation point should be investigated in future studies as the total number of stained bacteria and fungi alone do not suggest saturation of the collecting film, but they also do not indicate the total amount of non-biological particles loaded on REPS. The negative correlation between REPS and the Button Sampler collection for total bacteria and fungi may have been caused by overloading of REPS by non-biological particulate matter. Conversely, the continuous loading of non-biological particulate matter onto the Button Aerosol Sampler filters may have contributed to inactivation and sampling stress on culturable microorganisms. This may have partly contributed to preservation of a positive correlation between the performances of the Button Aerosol Sampler and REPS for culturable microorganisms and an overall improved sampling performance for REPS relative to the Button Samplers for collection of culturable bacteria.

4.6 Conclusions

In conclusion, I describe successful testing of a novel tool for bioaerosol exposure assessment in highly varied meteorological conditions with broad applicability to different sampling environments. REPS can significantly improve the field of bioaerosol sampling by allowing for easier, more cost-effective sampling over wider spatiotemporal scales, and less burdensome personal sampling as no pumps are needed. The results suggest that REPS is particularly well suited to passive sampling for long term monitoring of culturable bacterial aerosols with an equivalent sampling rate comparable

to active samplers (~2,600 mL/min). While more research is needed to understand the predictability between REPS performance and active reference samplers, its value is that is has a high collection efficiency through passive sampling means allowing to quickly reach detection limits for microbial analyses. With improved bioaerosol collection efficiency and capability to collect the full size spectrum of bioaerosol particles(Therkorn et al., 2016a), REPS is the only passive bioaerosol sampler with evidence that viral, bacterial and fungal-sized species of concern may be collected. The capability of REPS to better preserve bacterial culturability can be a major benefit as viability is a key determinant for bioaerosol infectivity(Cox and Wathes, 1995). Potential applications of interest for the exposure science community include: epidemiological research with widespread area or personal monitoring, studying aeroallergen/pollen patterns, investigating sources of Legionella across a cityscape, citizen science programs or microbiome studies. Future studies will investigate REPS saturation point, how to more efficiently concentrate biological particles after they are extracted from REPS, and how to best adapt REPS for personal sampling.

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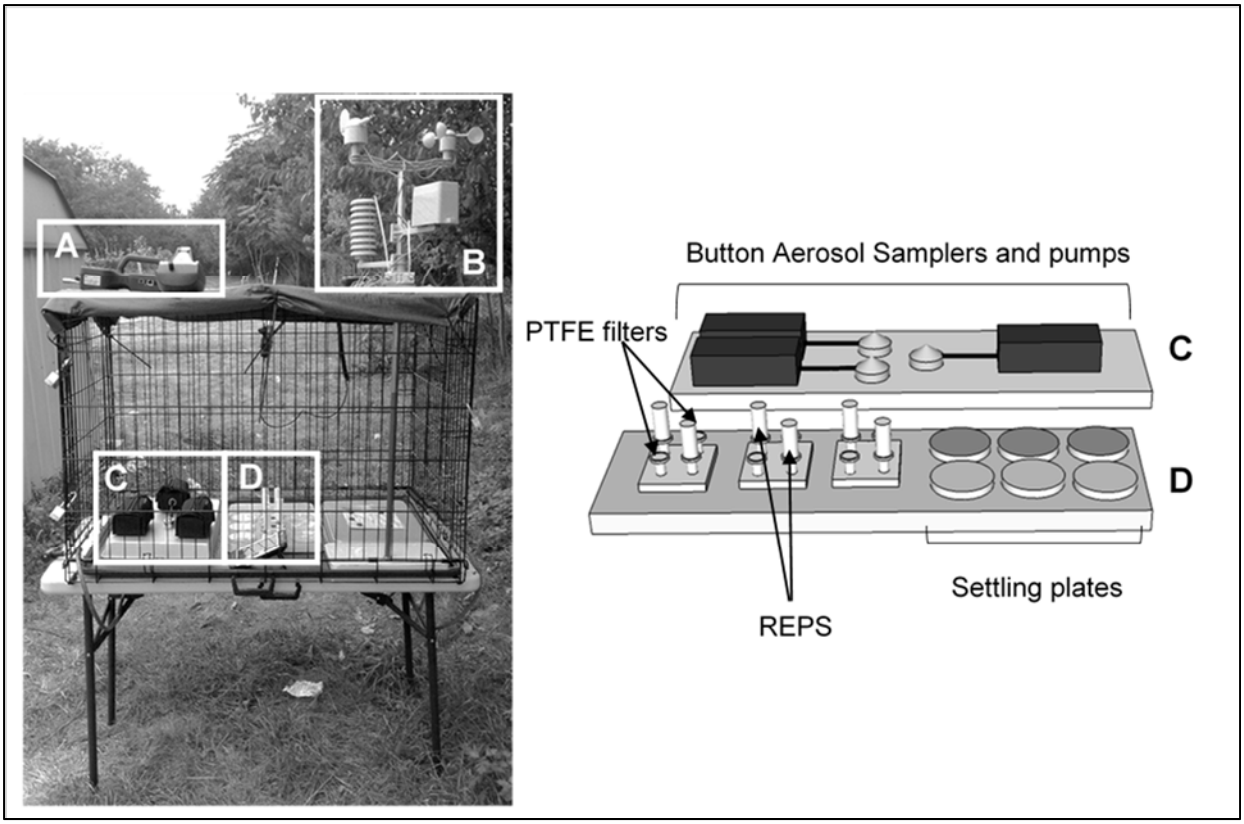

Figure 4-1.

The outdoor sampling site setup. 4-1A are the real time aerosol monitoring instruments operated each day for one hour, including the Aerotrak Handheld Optical Particle Counter (OPC, model 9306, TSI Inc., Shoreview, MN), and a DustTrak DRX Aerosol Monitor (DRX, model 8534, TSI Inc.). 4-1B is the portable weather station (WS-2080 Wireless Weather Station, Ambient Weather, Chandler, AZ). 4-1C are the active reference samplers for bioaerosol sampling operated in 24 hour intervals: three Button Aerosol Samplers (SKC Inc., Eighty Four, PA) operated at 4 L/min with PTFE filters (2 μm pore size, SKC Inc.). 4-1D are the passive samplers: six PTFE 25 mm settling filters, six REPS, and six 90 mm agar settling plates (three for culturable bacteria collection and three for culturable fungi collection).

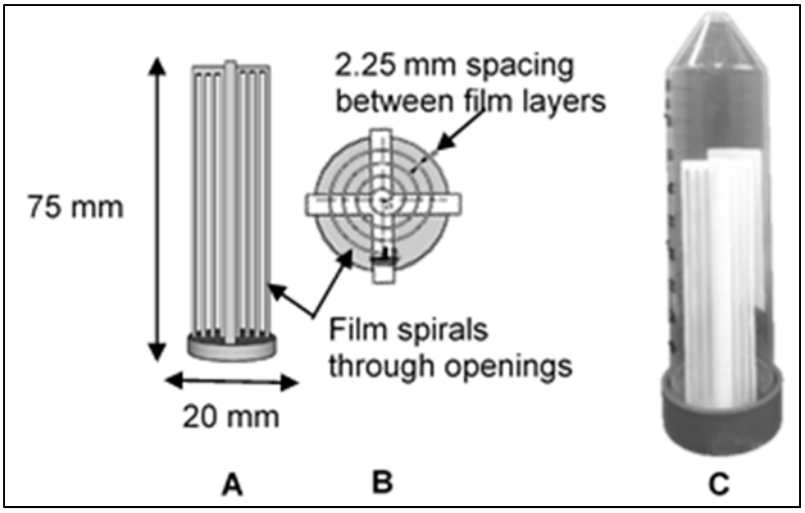

Figure 4-2.

Front (A) and top (B) views of the spiral 3D-printed REPS film holder, and REPS in a 50 mL conical centrifuge tube (C). One piece of film 130 x 70 mm is spiraled through the openings to form multiple layers of the film with sides of opposite polarization facing each other across 2.25 mm wide air channels.

Table 4-1.

Summary of Meteorological Conditions During Field Test Campaigns* (Data show mean ± 1SD, (Min, Max))				
Dates	9/20/15 – 9/29/15	11/1/15 – 11/11/15	11/14/15 – 11/24/15	12/13/15 – 12/23/15
Temp (°C)	19.72 ± 4.37, (10.00, 32.50)	14.4 ± 5.55, (-0.11, 27.39)	10.12 ± 4.80, (0, 21.28)	9.32 ± 5.23, (-2.28, 21.11)
Relative Humidity (%)	66.47 ± 19.67, (17, 98)	74.86 ± 21.64, (23, 99)	60.61 ± 20.56, (23, 99)	72.43 ± 20.55, (34, 99)
Wind Speed (m/s)	0.45 ± 0.47, (0, 3.40)	0.46 ± 0.61, (0, 4.11)	0.66 ± 0.80, (0, 5.41)	0.50 ± 0.65, (0, 4.38)
Peak Wind Gust (m/s)	0.86 ± 0.76, (0, 5.81)	0.87 ± 0.99, (0, 5.81)	1.30 ± 1.34, (0, 8.18)	1.13 ± 1.18, (0, 8.49)
Total Precipitation (mm)	0	16.76	21.34	38.54

* All meteorological data, except total precipitation, represent five minute averages of continuously recorded data throughout each sampling campaign. Total precipitation data from observations at a nearby remote weather station, The Rutgers Gardens Weather Tower, located about 1200 m away from the sampling site.

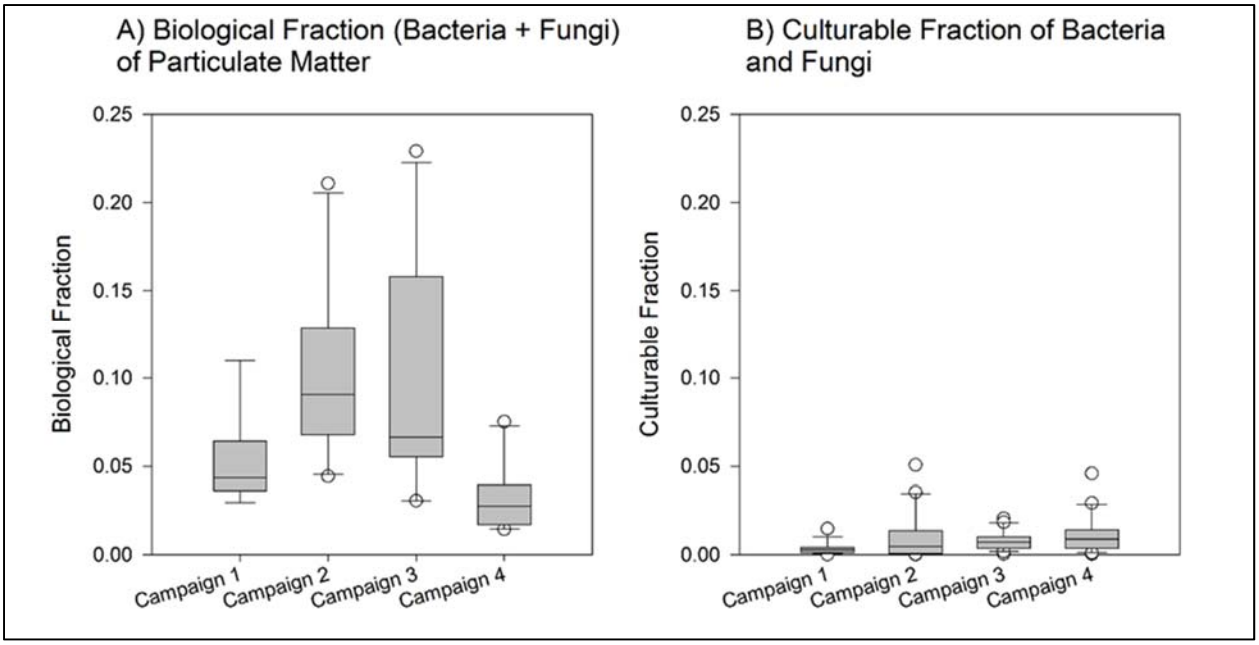

Figure 4-3.

The biological fraction (average daily totals for bacteria + fungi) of particulate matter > 1 µm (A) and the culturable fraction of those bacteria and fungi (B) for all sampling campaigns. An Aerotrak Handheld Optical Particle Counter (OPC, model 9306, TSI Inc., Shoreview, MN) monitored particle number concentrations in the 0.3 to 10 µm size range. This data was used to estimate the daily biological fraction of particulate matter by dividing the average total number of stained bacteria and fungi obtained from daily Button Samplers by the average total number of particles larger than 1 µm registered by the OPC across a given sampling campaign. Single and agglomerated bacterial and fungal particles are typically expected to be in the size range of 1 to 10 µm. Since the OPC was only run for 60 minutes each sampling day, the daily OPC values were averaged across each campaign to reduce variability in calculated daily biological fractions.

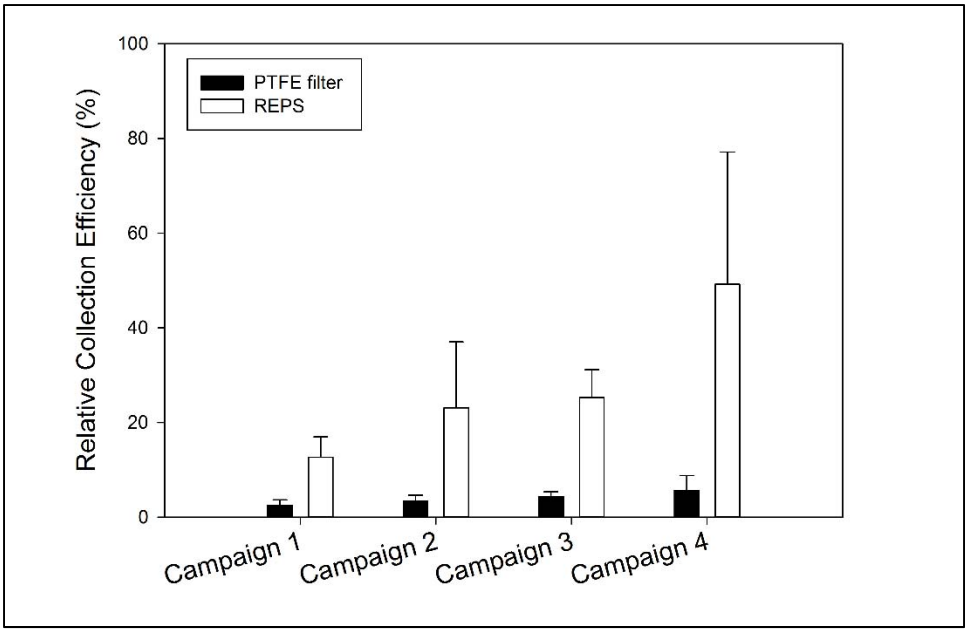

Figure 4-4.

Collection efficiency (%, mean ± 1SD) of total bioaerosol (bacteria + fungi) by passive samplers relative to the average total collection by Button Aerosol Samplers operated at 4 L/min. Each sampling campaign was 10 days long.

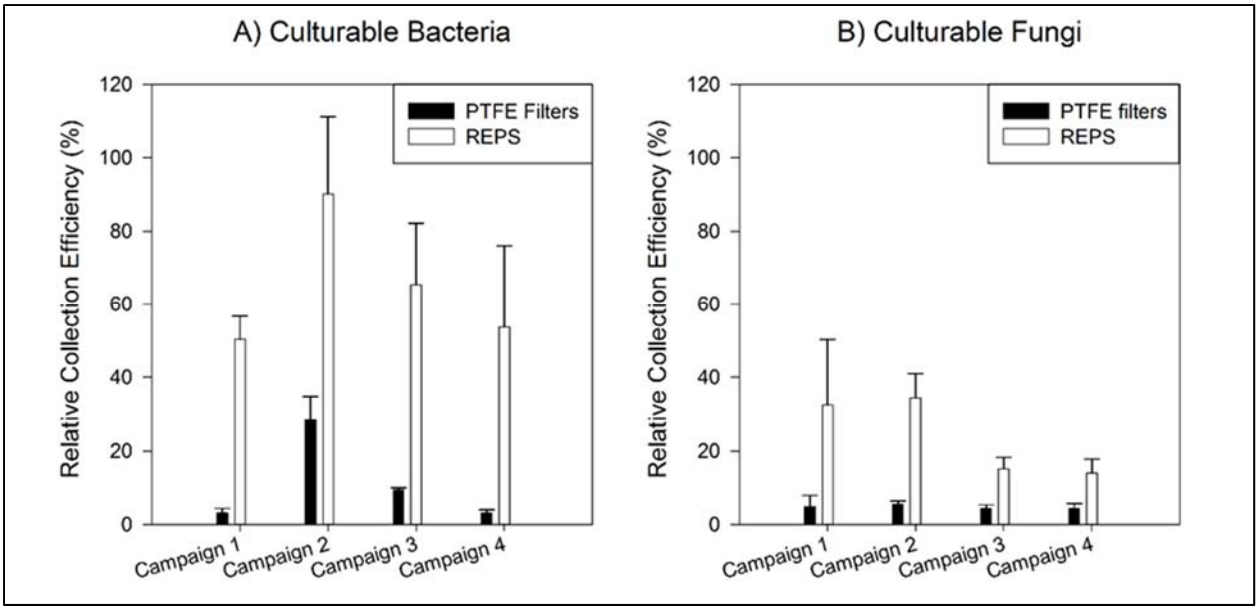

Figure 4-5.

Collection efficiency (%, mean ± 1SD) of culturable bacteria (A) and culturable fungi (B) by passive samplers relative to the average culturable bacteria and fungi collection by Button Aerosol Samplers operated at 4 L/min. Each sampling campaign was 10 days long.

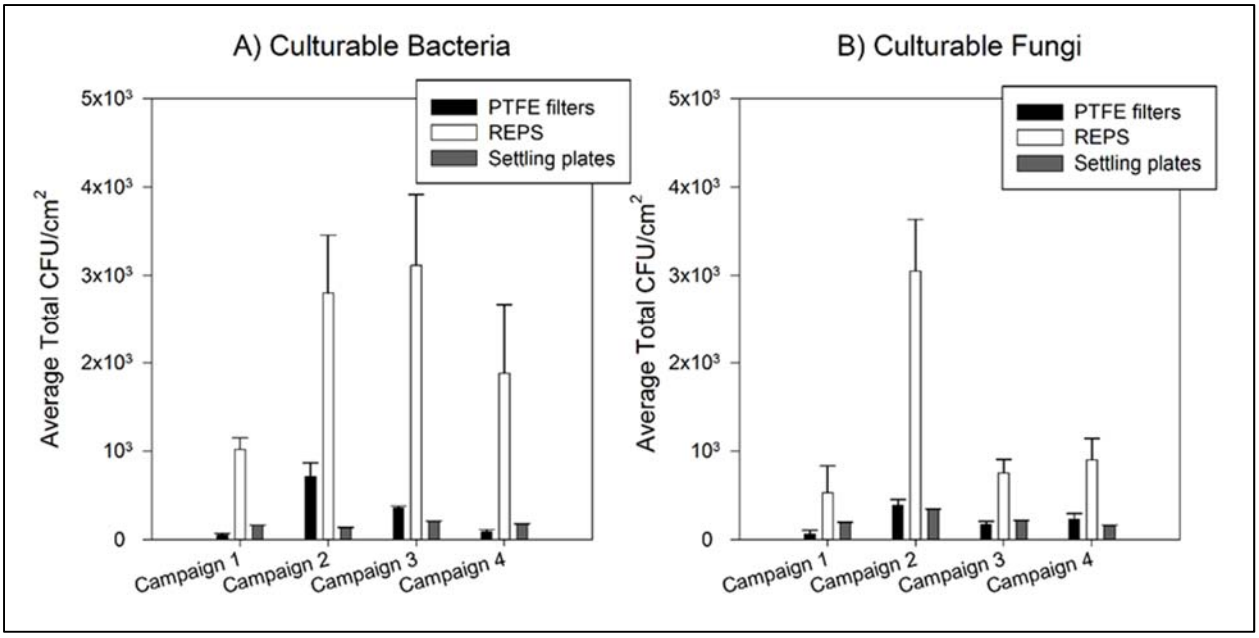

Figure 4-6.

Average total CFU normalized to sampler's surface area (CFU/cm2, mean ± 1SD) of culturable bacteria (A) and culturable fungi (B) collected by passive samplers. Each sampling campaign was 10 days long.

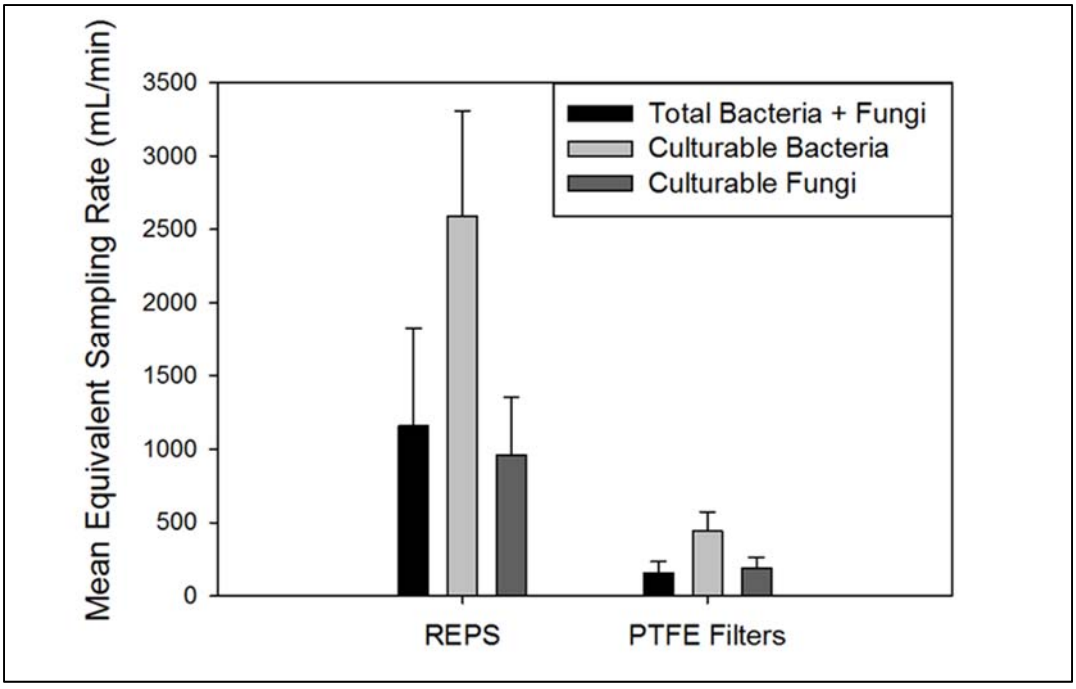

Figure 4-7.

Average equivalent sampling rates (mL/min, mean ± 1SD) for passive samplers compared to the Button Aerosol Sampler operated at 4 L/min. These values were obtained as the average across the four 10 day long sampling campaigns.

CHAPTER 5. SUMMARY, IMPLICATIONS, AND FUTURE DIRECTIONS

5.1 Summary

The main objective of this dissertation was to present the design, development and evaluation of a novel passive bioaerosol sampler. The novelty of the sampler is established through the incorporation of a polarized, ferroelectric polymer film (PVDF) with a 3D-printed film holder to enhance electrostatic capture of charged bioaerosol particles. The four main stages of passive sampler development and evaluation are presented throughout the three main chapters of this thesis and are as follows: 1) conceptual design, 2) extraction efficiency testing, 3) prototype design and testing, and 3) outdoor field evaluation. Collectively, these developmental stages address the four specific aims of the dissertation.

5.1.1 Sampler Conceptual Design (Specific Aim 1)

The first specific aim was to conceptually design the passive sampler using parallel layers of a polarized, ferroelectric polymer film by determining the film type and air channel width that optimizes collection efficiency of microorganism-sized particles (approximately 0.014 to 5 μm). The use of 2.25 mm wide air channels between parallel layers of PVDF provided the greatest overall collection efficiency across the tested particle size range. The PVDF film was also found to be a strong, flexible film which made it ideal for sampler development. The enhanced particle capture was due to the polarization of the PVDF which results in an electric field between the parallel layers of film. These results provided a proof of concept and support for the theory of this dissertation that the electric field could be used to significantly enhance electrostatic capture of microorganism sized particles.

Furthermore, these tests showed that PVDF with 2.25 mm wide air channels could be used to capture particles carrying high surface charge, as well as particles exhibiting Boltzmann equilibrium charge distribution – the expected particle charge conditions during ambient sampling (Hinds, 1982). Collectively, these results provided the sampler's conceptual design. These experiments served as the preliminary stage before designing field-deployable prototype samplers.

5.1.2 Extraction Efficiency Testing (Specific Aim 2)

The second specific aim was to determine the extraction efficiencies of spiked microorganisms from the surface of the polarized, ferroelectric polymer film type chosen through Specific Aim 1. The investigated extraction procedure of vortex mixing followed by ultrasonication was chosen since it has been previously shown to be time efficient and effective (Wang et al., 2001), and could be conveniently conducted with the samplers contained in standard 50 mL conical centrifuge tubes. 100% extraction efficiency from the PVDF film and 3D-print materials was achieved for all tested microbes (two bacterial species and one type of fungal spore), and these extraction efficiencies were statistically significantly greater than that achieved from the two control materials (PTFE filters and ESDs). Thus, PVDF was determined to be a prime candidate for designing the passive sampler prototypes as it was found to provide not only greater collection efficiency of bioaerosol sized particles, but also significantly improved recovery of collected microbes for post-sampling analyses. The nylon 3D-print material was chosen for prototype production as the lower melting temperature of the acrylic material would not be appropriate for beginning field tests outdoors during summer.

5.1.3 Testing of Field Deployable Prototype Sampler Designs (Specific Aim 3)

The third specific aim was to compare collection efficiencies of mixed bacteria aerosol of field-deployable prototype sampler designs using a compact, calm air settling chamber. Two types of sampler prototype designs were investigated – a spiral and a lattice. The integration of poled PVDF film with the prototype designs provided a greater than six-fold increase in the number of captured bacteria compared to gravimetric settling onto a reference PTFE filter with similar projected surface area. This improved collection efficiency was statistically significantly greater than that provided by the film holders only (no film) and the film holders with unpoled PVDF film. As there were no statistically significant differences in the prototype design performances, the spiral design was chosen to progress to outdoor field testing because it was easy to lace the film into the sampler's openings, and it held the film securely. Overall, key evidence was provided for the proof of concept in using the prototype passive sampler to significantly enhance the capture of bioaerosol particles compared to passive gravitational deposition alone.

5.1.4 Outdoor Field Testing (Specific Aim 4)

The fourth and final specific aim was to conduct outdoor field testing to evaluate the performance (equivalent sampling flow rate) of the optimized passive sampler design versus active and passive sampler controls. Prior to conducting the field campaigns, a sampler trade name was chosen: the Rutgers Electrostatic Passive Sampler (REPS). Field campaigns were conducted across highly varied weather conditions from September to December so that the research could be applicable to sampling environments and conditions other than the one chosen for this study. Compared to passive PTFE filters, REPS enhanced passive deposition of total microorganisms by 7-fold, and collected 65% of the culturable bacteria that the active Button Samplers collected. Since the Buttons

operated at 4 L/min, REPS had an average equivalent flow rate of 2.6 L/min and 1.0 L/min for culturable bacteria and total bacteria + fungi, respectively – about an order magnitude greater than that of the PTFE filters. These resultant equivalent sampling rates for REPS are also much greater than equivalent sampling rates reported for previously designed passive aerosol samplers (Table 5-1). These results suggest that REPS passively collects microorganisms comparably to an active sampler over long sampling durations, especially for culturable bacteria. This is due to high collection efficiency and better preservation of microorganism culturability in the absence of a desiccating flow rate.

5.1.5 Evaluation of REPS Performance

The hypothesis for this dissertation was that polarized, ferroelectric polymer films could be used to design a passive bioaerosol sampler that met the following *final sampler goals*:

1. Collection of the full size spectrum for bioaerosol particles, ranging in size from the nano-scale, which includes single viral particles, to the micron-scale which includes bacteria, fungi, pollen, and larger biological/non-biological particulate matter agglomerates;
2. Statistically significantly greater extraction efficiency of collected microorganisms as compared to control materials typically used for active and passive bioaerosol sampling.
3. Significantly greater passive bioaerosol particle capture as compared to passive deposition controls; and,
4. An equivalent sampling rate calibrated against an active reference control sampler statistically significantly greater than 100 mL/min (the approximate equivalent

sampling rate of the Personal Aeroallergen Sampler and the highest reported value for a previously developed passive aerosol sampler (Yamamoto et al., 2011)).

As there are currently no set standard metrics for passive aerosol or bioaerosol sampler performance, these final sampler goals were chosen as the benchmarks of success for this dissertation. The first goal was met as is evidenced by the results of Specific Aim 1. Test particles ranging in size from 0.014 to 5 μm were captured with statistically significantly greater collection efficiency when using PVDF with 2.25 mm wide air channels as compared to non-ferroelectric controls. Thus, it can be expected that this film/air channel orientation will provide collection of the full size spectrum for bioaerosol particles. As of yet, this performance criterion is not met by all other passive bioaerosol samplers which rely on gravitational settling of particles.

The second goal was met as is evidenced by the results of Specific Aim 2. Statistically significantly improved extraction efficiency was observed from the surface of PVDF (and its 3D-printed film holder material) as compared to control materials typically used for active and passive bioaerosol sampling. ~100% extraction efficiencies of bacterial and fungal microbes were found for PVDF.

The third goal was met as is evidenced by the results of Specific Aim 3 and outdoor field testing. Using the calm air settling chamber to compare the capture of a settling mixture of bacteria by prototype samplers versus reference PTFE filters, it was found that the prototype sampler with poled PVDF provided statistically significantly greater bacterial capture over all tested film conditions regardless of prototype design (lattice vs. spiral). During outdoor field testing, compared to passive PTFE filters, REPS

provided statistically significantly greater passive capture of total and culturable microorganisms across all four campaigns.

The fourth goal was met as is evidenced by the results of Specific Aim 4. Outdoor field testing of REPS versus active reference Button Aerosol Samplers operating at 4000 mL/min showed that REPS had an average equivalent flow rate of ~2600 mL/min and ~1000 mL/min for culturable bacteria and total bacteria + fungi, respectively. The achieved equivalent sampling rates of REPS meet and surpass the fourth goal. The equivalent sampling rates of REPS were statistically significantly greater than that of PAAS, and were about an order of magnitude greater than the equivalent sampling flow rate determined for PAAS as well as the reference PTFE filters.

REPS provides the benefits of typical passive samplers, like compact size, portability, and low-cost. With a focus on user experience and design thinking, other benefits provided by REPS include streamlined handling procedures as the sampler has been designed to fit into standard 50 mL conical centrifuge tubes. This simplifies transport to and from the field, minimizes contamination, and allows for convenient particle extraction prior to analyses. As the sampler design is based on thin, flexible films, REPS is also fully customizable to any sampling application by adjusting the size, shape or configuration of the films and film holder.

Table 5-1 presents passive bioaerosol and aerosol samplers which have been previously described in the literature. The inclusion criteria for the passive samplers in this table are: 1) the sampler is presented in the literature with the purpose of passively collecting airborne particles (biological and non-biological), and 2) the sampler is referenced in the literature more than once. It is apparent that inter-comparability of these

currently available passive aerosol samplers is limited by the variability in the chosen methods for particle capture, analyses and evaluation. This calls for the need for better standardization in the development of passive aerosol sampler technology. Equivalent sampling rates have most likely not been calculated for other samplers due to the low collection efficiency of these samplers versus their active reference controls; thus, it was most likely not appropriate for these samplers to be compared on the same scale as the active reference samplers. It is also apparent that there are few currently available passive aerosol samplers which have been designed specifically for bioaerosol particle collection. Comparatively, REPS has been designed specifically for bioaerosol sampling, allows for post-sampling analyses to be conducted with any microbiological method, has been shown to capture particles between 14 nm and 5 μm, and has the highest reported equivalent sampling rate.

5.2 Potential Applications for REPS Technology

REPS technology is flexible to be customized for both area and personal sampling applications. Area samplers can be used individually or grouped as multiples in a unit for *in situ* monitoring to enable detection of biological hazards in specific locations. Area samplers can be designed to have accessories incorporated for mobile applications that utilize air movement, such as a wind vane, or onto vehicles, like city buses, for a mobile sensor network. The sampler could also be incorporated with unmanned aerial vehicles (UAV's, i.e., drones) to investigate, for example, bioaerosol role in atmospheric chemistry as a function of altitude and meteorological profile (Martin et al., 2011). Personal samplers can be designed to be worn by individuals in the performance of their duties, such as first responders or healthcare workers (similar to a radiation badge).

The unique advantages of the proposed sampler will translate into broad applications like infectious disease monitoring, global climate change research, agricultural protection, occupational health and safety sampling, increased capacity for community outreach, and homeland security. The ability to better monitor airborne infectious disease could lead to better management of healthcare facilities – a current need for protecting staff and patients and reducing hospital acquired illnesses (Clark and de Calcina-Goff, 2009). The airborne route of infectious disease transmission is also an increasing concern due to the emergence of extensively drug resistant bacteria, like *Mycobacterium tuberculosis* (Cegielski, 2010).

Since the sampler will be remotely deployable and capable of providing fine spatial resolution, it will meet current needs for obtaining sentinel information on fungal threats to crops (Jackson and Bayliss, 2011) and improving global climate change study. Global climate change will continue to impact health by impacting patterns of aeroallergen production, evolution of airborne pathogens and potential for bioaerosol exposures through changes in land use (Boxall et al., 2009; Cutler et al., 2010). Another related issue of particular relevance to the NY/NJ metropolitan area is the increasing threat of hurricane coastal flooding with consequent mold contamination (Hoppe et al., 2012; Mousavi et al., 2011). The proposed sampler may be an increasingly important tool for the future since it will be easier to assess bioaerosol exposures in emergency situations because it does not need batteries or external power.

Since a pump is not needed, the sampler can also increase capacity to collect representative long-term personal samples. For example, in studies on childhood asthma, there have been major limitations of collecting personal particulate matter data by

requiring the children to wear backpacks to accommodate sampler pumps (Keeler et al., 2002; Sagona et al., 2016). As a passive sampler, REPS technology is well suited for use by citizen scientists because it is easy to deploy, can be used anywhere, and does not have to be continuously monitored. Ultimately, by delivering a more versatile, portable and cost-effective bioaerosol sampler, the scope and spatial resolution of bioaerosol and other particulate pollutant monitoring will be expanded.

5.3 Implications

5.3.1 Practical Implications

Increasing capacity to collect air pollutant data fosters not just human health protection but also all other aspects of sustainable development like quality of life and natural resource protection (Knox et al., 2012). Issues related to the health impacts of bioaerosols have been largely irresolvable thus far due to limitations of current bioaerosol sampling methods. By providing a low cost option to complement higher volume active air sampling, passive samplers can augment sampling network data to meet the needs and challenges present for reporting temporal pollutant trends (Klánová and Harner, 2013). Sustainability requires reduction in energy consumption through widespread adoption of energy-saving measures (Hammond, 2004). Towards this end, a passive sampler like REPS can provide comparable bioaerosol sampling performance to active aerosol samplers without the need for external power.

The versatility and cost-effective operation of the sampler may also support establishment of protective health-based occupational exposure limits for which there are only a poorly defined few in the United States including dusts, like cotton and wood dusts, and one species-specific bacterial enzyme (Eduard et al., 2012). Better worker

protection is needed not only in the U.S., but also worldwide; but, establishment of new bioaerosol standards will not be feasible until the available sampling technology can allow for defining and regulating these standards (Eduard et al., 2012).

Political capacity to address environmental issues, such as inequitable distribution of health burdens and occupational exposure limits, partly depends on the scientific evidence available; therefore, the ability to collect relevant monitoring data helps determine which problems can be solved (Knox et al., 2012). This dissertation contributes to the greater scientific community as it provides a novel tool for bioaerosol sampling with unique advantages. By allowing for novel bioaerosol sampling applications, like long term and large scale sampling campaigns, new data will become available to analyze. In the world of policy making, scientific advancement must balance the needs between economic growth and environmental protection; in the world of environmental science, the work that is done ultimately affects not only policy, but how society responds to environmental challenges (Kriebel et al., 2001). Overall, the passive sampler will protect human health by allowing for more effective indoor/outdoor health-relevant bioaerosol monitoring and study, development of exposure limits, environmental/agricultural protection, and bioterrorism surveillance.

5.3.2 Methodological Implications

This dissertation not only provides a tangible, novel sampling tool, it also provides a new area of discovery for bioaerosol sampling technology. Polarized, ferroelectric polymer films have never been applied to particulate air sampling. Other samplers may be developed, such as active, electrostatic precipitators (ESPs), by integration of REPS sampling technology with air movers, like fans or pumps. ESP

technology is particularly of interest for sampling and air cleaning of ultrafine particles. ESPs have been tested as an in-duct solution for removal of ultrafine particles, but ozone generation was high during continuous operation (up to ~80 ppb) (Poppendieck et al., 2014) – REPS technology, for example, could be integrated with commercial brand home air filters to provide increased air cleaning for ultrafine sized particles as a pseudo-active sampler.

The methodology used to develop the sampler, from conceptual design, prototype testing, to field evaluation, may serve as a framework for future research developing passive or active bioaerosol sampling technology. Currently, there are no set standards for how to develop or evaluate any active or passive bioaerosol samplers (Duquenne et al., 2013; Dybwad et al., 2014). The research steps followed in this dissertation were based on the steps outlined by the initial research of Wagner and Leith during development of the UNC Passive Sampler. Briefly, their initial steps included: 1) description of the principle of operation for their sampler (Wagner and Leith, 2001b); 2) wind tunnel experiments (Wagner and Leith, 2001c); and, 3) field testing of the passive sampler to investigate results versus an active reference sampler (Wagner and Leith, 2001a).

The research presented in this dissertation offers several key improvements over the methodology chosen by Wagner and Leith. First, the addition of the calm air settling chamber allowed for investigation of how settling particles would be diverged from their settling paths by the passive sampler without the influence of forced air. The majority of indoor and outdoor environments have low air flow rates similar to the conditions within

a calm air chamber (Baldwin and Maynard, 1998; Feather and Chen, 2003; Sleeth and Vincent, 2012).

Second, field testing for this dissertation was conducted over four 10 day long campaigns with varied outdoor conditions to evaluate the applicability of the achieved results to sampling environments other than the one chosen for this study. Wagner and Leith (2001a) conducted five sampling events in a single occupational environment (glass manufacturing plant), and the total combined length of their sampling events was less than 24 hours. This limits interpretation of results beyond the scope of their particular research setting.

Third, by presenting an equivalent sampling rate for REPS during outdoor field testing, the results can be interpreted against known reference active samplers. By estimating an equivalent sampling flow rate, and by achieving such a flow rate comparable to typical active aerosol samplers, REPS may serve as a starting point for developing passive bioaerosol samplers that can provide quantitative data on bioaerosol concentrations. Future sampler development will benefit from the establishment of a new performance benchmark set by REPS during outdoor field evaluation.

5.4 Future Directions

Future research and directions will focus on designing and evaluating different types of samplers to be tailored to sampler applications of interest, as well as sampler commercial development. The application of ferroelectric polymer films will also be investigated for use in active bioaerosol sampling with air movers, like air pumps or fans. Also, there were several limitations observed during this research that should be addressed in future studies.

5.4.1 REPS Saturation Point

One limitation presented through the outdoor field testing was a moderate negative correlation between REPS and the Button Aerosol Sampler's collection of total bacteria and fungi (r: -0.40). The relationships between REPS and the active reference Button Aerosol Sampler's performances as depicted in Figure C-3 indicate that the high levels of particulate matter pollution may have caused REPS to reach a saturation point during testing. This saturation point should be further investigated in future studies as the total stained bacteria and fungi alone do not indicate the total amount of non-biological particles loaded on REPS.

To conduct initial investigations on the REPS saturation point, further laboratory testing is recommended using high concentrations of mixed biological and non-biological test particles. For example, these tests could be performed in the calm air settling chamber with highly concentrated particles, including a slurry mixture of Arizona Road Dust and bacteria to simulate different ratios and concentrations of biological and non-biological particulate matter.

Further field testing of REPS is also recommended. A group of REPS samplers can be deployed and several samplers can be returned to the lab on each testing day to investigate how the collection efficiency of REPS changes over time versus reference Button Aerosol Samplers. The apparent saturation point of REPS may be related to not only particle overloading, but also other environmental or meteorological factors.

5.4.2 Sample Concentration

A second limitation is that a relatively large volume of extraction fluid (40 mL) is needed to fully submerge REPS in the 50 mL conical centrifuge tubes for particle

extraction by ultrasonication. For comparison, a 25 mm diameter filter only requires about 5 mL of extraction fluid to be submerged. For the research presented in this dissertation, bioaerosol concentrations were sufficiently high so that it did not hinder staining and counting by epifluorescence microscopy. However, to conduct sampling for shorter time periods or in environments with lower particulate levels, or to use different types of microbial analyses, future research should investigate sample concentration. Potential methods for doing so include reducing the volume of extraction fluid, and/or efficiently concentrating the sample post-washing.

Methods which may be used to reduce the volume of water used in the extraction procedure include the use of a wrist motion shaker to wash particles from REPS surface (Burton et al., 2005). By orienting the conical centrifuge tube horizontally with REPS inside, the shaker can agitate the extraction suspension to fully wash the sampler while not requiring the sampler to be fully submerged. Another possibility is to investigate how to concentrate the sample after washing using a new Concentrating Pipette (InnovaPrep, Drexel, MO). This automated instrument provides mechanical enrichment of a liquid sample containing biological particles by filtering the sample, and then extracting the filter with a smaller volume of liquid. This procedure has several main drawbacks. First, the extraction fluid contains detergents to aid in particle removal from the filter; this is known to negatively impact microbial integrity (Zhen et al., 2013). Second, the procedure is resource intensive as it requires specialized, disposable items, like the filtering pipettes, and extraction suspension canisters.

Other options for sample concentration include development of a filtration system to easily filter the large volume of extraction suspension. Typical filtration systems, like

the laboratory filtration system #3 of the Millipore company which is designed to run more than 20 samples per day (Millipore Corporation, Darmstadt, Germany), require slowly running liquid samples through the filters so that the filters are not damaged by excessive vacuum pressure. Thus, filtering of multiple 40 mL samples could be time consuming. Furthermore, unless directly staining onto the filter for microscopic analysis, the particles will have to then be removed from these filters for other types of analyses. Centrifugation to concentrate samples is also a possibility, but as the extraction suspension is 40 mL, centrifugation would be most effective by aliquoting the suspension into 1.5 mL Eppendorf tubes for micro-centrifugation. Again, this can be a time consuming procedure. Thus, I recommend the wrist-motion shaker, or another similar agitation technique, for the initial steps of investigating sample concentration.

5.4.3 Sampler Commercialization

The final future direction is sampler commercialization. Currently, two provisional patent applications have been submitted with application focus areas of passive and active sampling:

1. Passive Particulate Sampler using Ferroelectric Polymer Films. U.S. Provisional Application 62/222,857, filed September 24, 2015. Patent Pending.
2. An Electrostatic Precipitator using Poled, Ferroelectric Polymer Films. U.S. Provisional Application 62/334,118, filed May 10, 2016. Patent Pending.

Commercial development of REPS sampler technology through collaboration with the Rutgers Office of Research Commercialization will help to ensure that the sampler is actually utilized for the applications in which it is needed most.

5.5 References

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Table 5-1

Comparison of REPS with Other Passive Bioaerosol/Aerosol Samplers

Sampler	Sample Type and Analysis Method	Particles Collected	Evaluation metric
REPS	Bioaerosol or general aerosol (any microbiological method)	>0.014 μm	Equivalent sampling rate = 1 L/min for total and culturable fungi, 2.6 L/min for culturable bacteria
Settling Plates (Cox and Wathes, 1995)	Bioaerosol (culturable)	Unknown	Yes or no for presence of microorganisms
Electrostatic Dust Cloth (Kilburg-Basnyat et al., 2015)	Endotoxin (LAL assay) conducted in literature; can be used with any microbiological method	Unknown	Correlation (R^2 = 0.7 compared to Button Sampler)
PAAS (Yamamoto et al., 2006)	Allergen and pollen by gravitational settling – particles > 5 μm (microscopy/deposition modelling)	>10 μm	Equivalent sampling rate = 32 ± 6 mL/min to 66 ± 44 mL/min when sampling fungal species > 5 μm in aerodynamic diameter
Wagner-Leith (Wagner and Leith, 2001; Wagner and Marcher, 2003)	Only tested for general aerosol (microscopy/deposition modelling)	PM10 (difficulty with PM2.5)	Correlation (R^2 = 0.7 compared to 8-stage impactor)
Einstein Lioy Deposition Sampler (Canha et al., 2014; Einstein et al., 2012)	Only tested for general aerosol (microscopy/deposition modelling)	>1 μm	Trend comparison (Chi Square goodness of fit: p = 1)
Electret (Brown et al., 1996; Brown et al., 1995; Hemingway et al., 1997; Thorpe et al., 1999)	Only tested for general aerosol (mass concentration relative to active sampler)	PM10	Equivalent sampling rate = 22.5 mL/min when testing its performance in the laboratory to collect asbestos fibers
Sticky foils (Schneider et al., 2002; Vinzents, 1996)	Only tested for general aerosol (light extinction on foils)	> 100 μm	Correlation (R^2 = 0.8 compared to APS)

APPENDIX A: SUPPORTING INFORMATION FOR CHAPTER 2

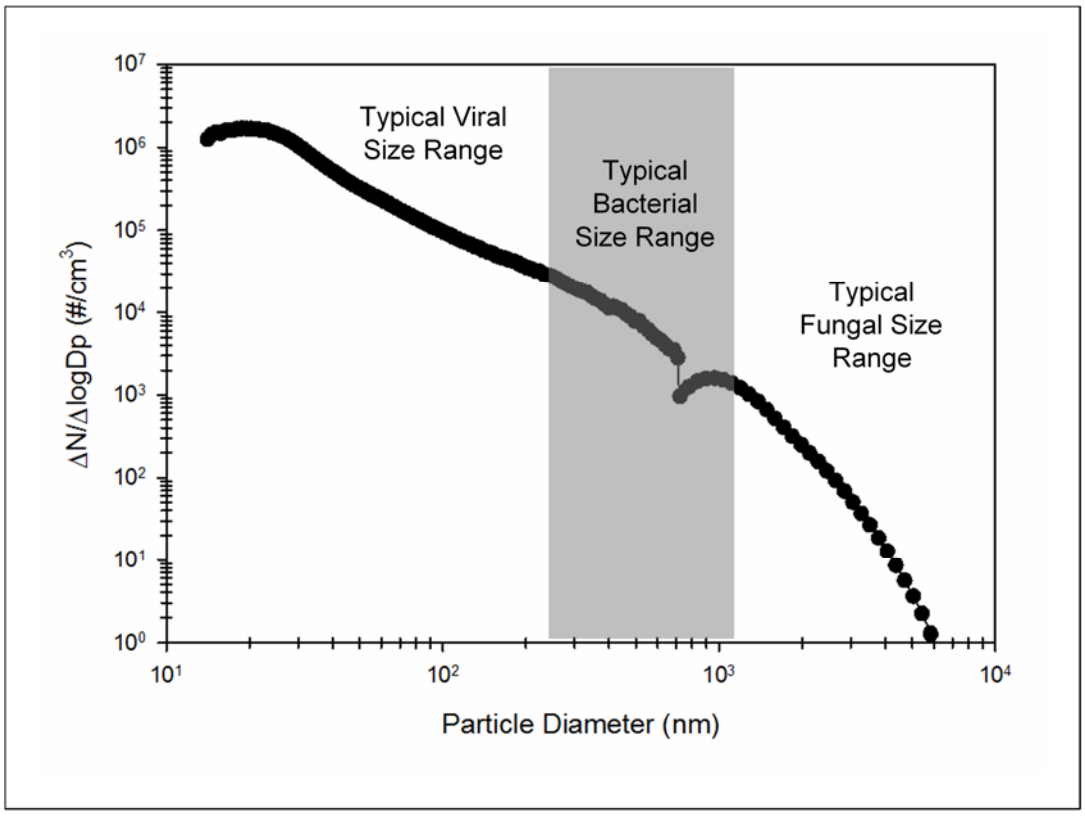

Figure A-1.

Particle number size distribution for Arizona Road Dust (ARD) slurry (25 mL) produced from 3-Jet Collison Nebulizer at 5 L/min, 20 psig. Measurements were made using the combination of SMPS (model 3080 with DMA 3081; TSI Inc., Shoreview, MN) and APS (model 3321; TSI Inc.). The shaded region indicates separation of typical size ranges for bioaerosol particles. Size ranges were designated based on the size bins of the SMPS and APS and typical sizes of biological particles: virus size = 14.1 to 250.3 nm, bacteria size = 259.5 to 1286 nm, and fungal size = 1382 to 5048 nm. The discontinuity in the particle number size range at about 700 nm is a result of where the data are brought together from the SMPS (measures 14 nm to 723 nm) and the APS (measures 500 nm to 20,000 nm) as the APS tends to underestimate particles near the minimum of its measurement size range

(Price et al., 2014). The typical bacterial aerodynamic diameter size range is from 1-3 μm in clean indoor environments and 0.5 to 10 μm in more contaminated environments (Gorny et al., 1999; Reponen et al., 2011). Fungal spores tend to have aerodynamic diameters of 2-10 μm (Yamamoto et al., 2014) with smaller fragments of fungal material, like glucans and mycotoxins, being smaller than 1 μm (Gorny et al., 1999; Reponen et al., 1994). Viruses are the smallest microorganism with physical sizes of about 20-300 nm (Blachere et al., 2009; Reponen et al., 2011).

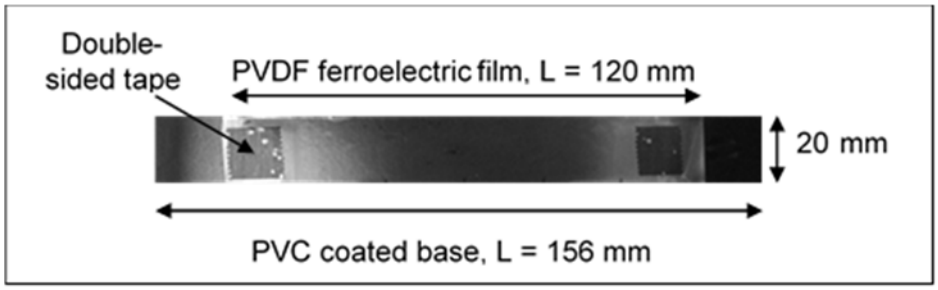

Figure A-2.

Photograph of the base (metal + PVC) with PVDF film affixed to it by double-sided tape. The black matte material covering the metal base is the PVC tape. The film did not cover the entire surface of the base to leave room for handling the bases while inserting them into the spacing lattices.

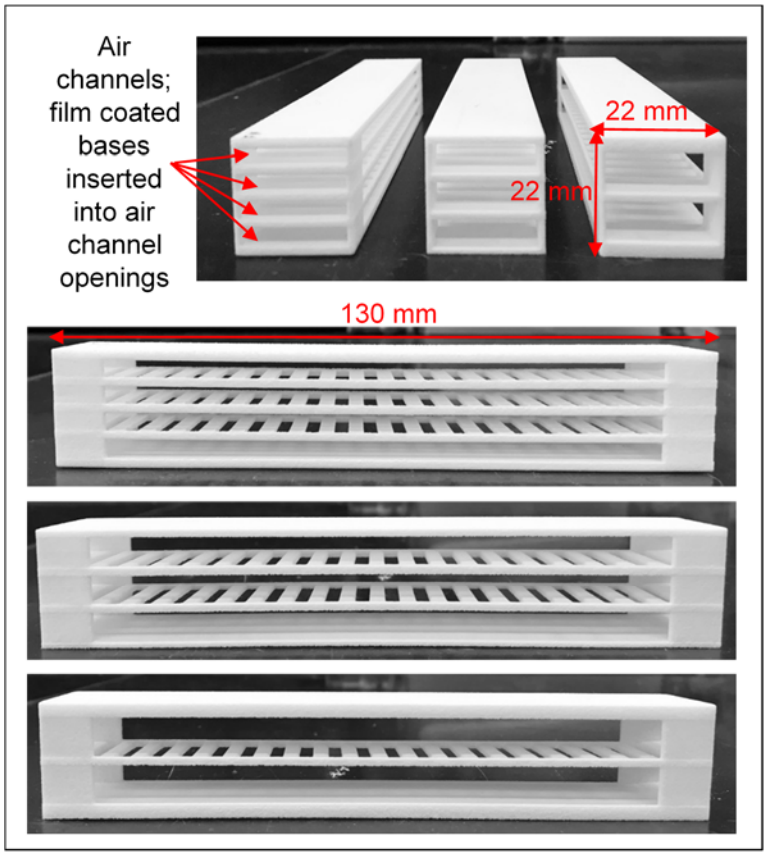

Figure A-3.

Photographs of the spacing lattices used in experimental setup of Figure 2-2. The top photograph shows from left to right the front view of the 2.25, 4.25 and 6.25 mm air channel spacing lattices. The bottom three photographs show side views of these lattices in the same order. The 1.00 mm air channel lattice was made by using the 2.25 mm lattice and decreasing the air channel width to 1.00 mm by increasing the amount of insulating PVC tape wrapped around the base. For testing the metal control in the 1.00 mm spacing lattice, three metal bases were glued together to achieve an air channel spacing distance of 1.00 mm.

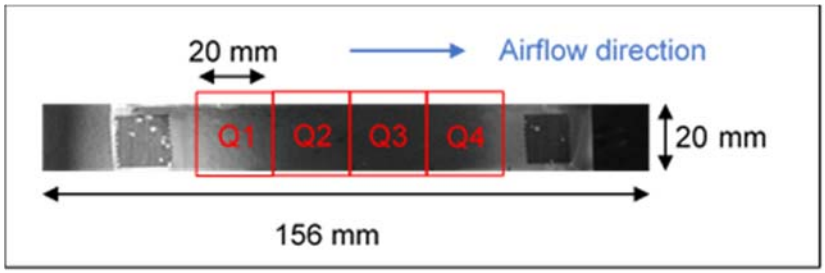

Figure A-4.

Photograph of the base (metal + PVC) with PVDF on top. The red boxes indicate quadrants Q1 to Q4 as positioned for experimental procedures described in material and methods section 2.3.6.

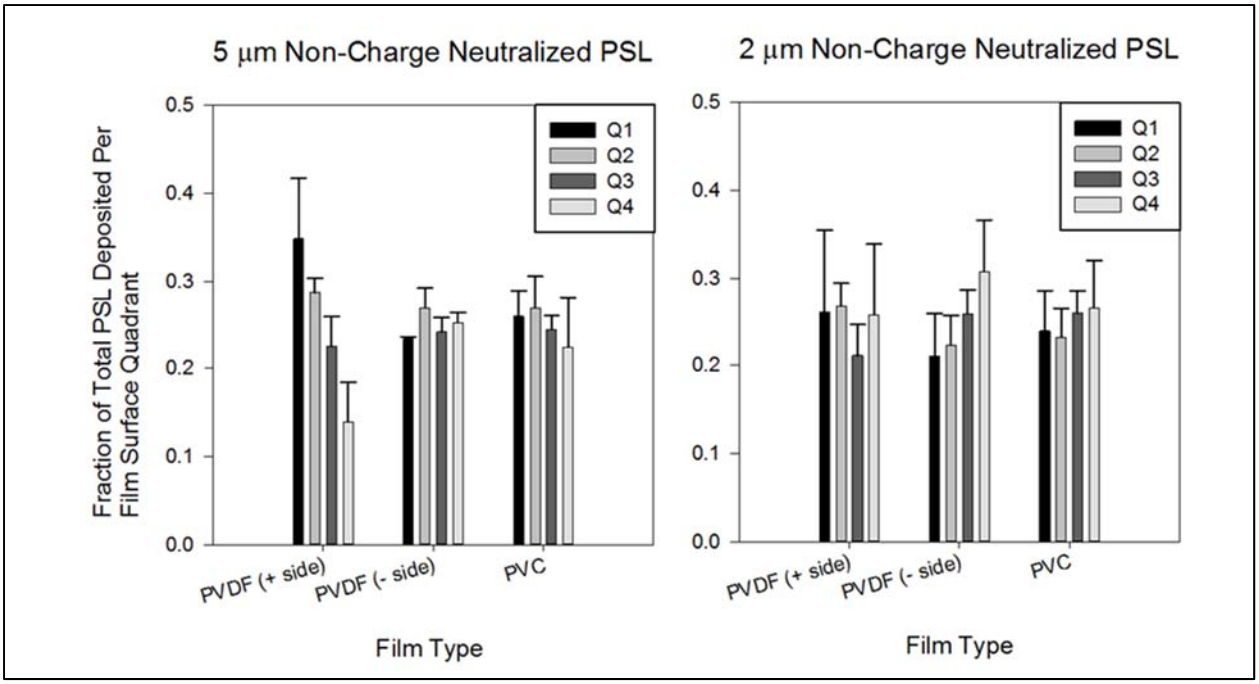

Figure A-5.

Deposition of non-charge neutralized PSL (2 and 5 µm) per quadrant on the surfaces of positive and negative polarization sides of PVDF films and PVC control films. The PSL particle counts per quadrant (Q1 to Q4) are represented as a fraction of the total particles on a film's surface. Q1 represents the front most quadrant of the film where particles entered the lattice.

Appendix A1. References

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APPENDIX B: SUPPORTING INFORMATION FOR CHAPTER 3

Appendix B1. Calm Air Settling Chamber Test Condition Measurements and Quality Control

The temperature and relative humidity throughout experiments was 20-25°C and 24-27%, respectively. For each experimental repeat, all surfaces on the inside of the chamber, including the rotating platform, were wiped with 70% ethanol. All tubing to instruments was as short as possible and conductive to minimize particle loss. Gloves were worn throughout experiments to minimize contamination. The system was too tall to put into a laminar flow hood, so a face mask, eye protection and lab coat were worn throughout experiments. Ports were installed in the chamber to allow for reference measurements to be taken prior to conducting any experiments to determine conditions that could be expected within the chamber during experiments. Two reference sampling ports were installed in the sampling area of the chamber 7 cm from the bottom, 90° apart. Eight reference ports were positioned in the aerosolization area of the chamber in a circular pattern; these holes were drilled into the top of the chamber about 5 cm from the chamber walls. During actual experimental repeats for prototype testing, these ports were sealed with airtight plugs.

Chamber condition measurements included particle number size distribution measured by an Aerodynamic Particle Sizer Spectrometer (APS, model 3321, TSI Inc.) and aerosol net charge measured by an Aerosol Electrometer (model 3068, TSI Inc.). Three repeat experiments were conducted to investigate particle number concentrations that could be expected in the chamber during experiments. For these repeat experiments, twenty second sample measurements were taken from the chamber every 15 min during

aerosolization (60 min total) and up to 150 min during settling time. The three test runs were averaged together to estimate the average aerosolized bacteria number concentration and net aerosol charge (current in electrometer) in the chamber during prototype testing (Figure 3-4). A Velocicalc velocity meter (model 9535-A, TSI Inc.) was inserted into the reference ports to verify calm air and mixing air conditions (Feather and Chen, 2003). These measurements were not conducted during prototype testing as the instruments sampling in the chamber would remove settling bacteria from the chamber and/or disturb the calm air conditions introducing further variability in prototype testing.

Aerosol homogeneity was investigated systematically by investigating progressively smaller polystyrene latex (PSL) test particles of 5.0 μm, 3.1 μm and 1.0 μm diameter (G0500, G0300, G0100, Thermo Scientific, Fremont, CA). The homogeneities of these aerosolized particles depositing across the chamber bottom were investigated using glass fiber filters (Type AE, 47 mm, Pall Corp., Ann Arbor, MI). These PSL particles were aerosolized individually as described in the methods with dilution factors of 5x10-2, 5x10-2, and 10-2 in sterile, Milli-Q water for the 1.0, 3.1 and 5.0 μm PSL, respectively. These particles were allowed to settle completely, and then the chamber was opened, the filters removed with forceps into glass jars of 4 mL ethyl acetate, and the PSL particles were allowed to dissolve completely for 180 min (3.1 and 5.0 μm PSL) or overnight (1.0 μm PSL). The relative mass concentrations of PSL particles depositing across the filters were determined as fluorescence intensity by analyzing the ethyl acetate liquid in a fluorometer (Sequoia-Turner Corp., Mountain View, CA) using a previously described procedure (Han and Mainelis, 2008). At least three experimental repeats were

completed for each particle size to determine the coefficient of variation for fluorescence intensity across the filters (Table 3-1).

From these initial experiments, it was determined that the rotating platform had to be incorporated, and so homogeneity of P. fluorescens bacteria depositing across the rotating platform was then investigated. Three PTFE filters were placed onto the rotating platform and P. fluorescens bacteria (4.06 x 108 cells/mL) were aerosolized as described in the methods. After the bacteria settled completely, the filters were removed, bacteria extracted, and total number of bacteria deposited onto each filter determined by staining/counting (also described in the methods). This experiment was repeated three times to estimate the coefficient of variation for settling bacteria onto the platforms three filters (Table 3-1).

Appendix B2. References

Feather, G. A. and Chen, B. T.: Design and use of a settling chamber for sampler evaluation under calm-air conditions, Aerosol Sci. Tech., 37, 261-270, 2003.

Han, T. and Mainelis, G.: Design and development of an electrostatic sampler for bioaerosols with high concentration rate, J. Aerosol Sci, 39, 1066-1078, 2008.

APPENDIX C: SUPPORTING INFORMATION FOR CHAPTER 4

Appendix C1. Calibration of the DustTrak DRX Aerosol Monitor (DRX, model 8534, TSI Inc., Shoreview, MN) to PM2.5 Personal Modular Impactors (PMIs, SKC Inc., Eighty Four, PA)

The DRX uses light scattering laser photometers to collect real-time aerosol mass data. The instrument is factory calibrated with the respirable fraction of standard ISO 12103-1, A1 test dust and so assumptions are made about the relationship between light scattering and mass concentration based on this test dust's particle size and material properties (TSI Incorporated, 2012). In order to calibrate the DRX aerosol monitor to the specific particulate matter characteristics of dust at the sampling site, two 24-hour sampling periods (randomly chosen days of the sampling campaigns) were conducted where the DRX monitor sampled at the site simultaneously with two PMIs. The impactors were operated with at 3 L/min using 37 mm PTFE filters (2 μm, SKC Inc.) and 25 mm pre-oiled impaction substrates (SKC Inc.). The filters used in the PMIs were weighed with a Mettler Toledo MT5 Microbalance before and after each sampling using a temperature and humidity controlled weighing room (20-23°C, 35-40% relative humidity). Filters were kept in their own cassettes before and after use and allowed to equilibrate in the weighing room for at least 72 hours before being weighed. Three filters were kept in the weighing room throughout all experiments to use as standards to weigh them every time before new mass measurements were taken to ensure consistency of results on different days. A 200 mg NIST standard was weighed prior to taking any mass measurements to also ensure consistency of results. The change in weight of each filter and total sampled air volume was used to calculate the average PM2.5 mass

concentration collected by the impactors over the 24 hour period and this data was used to obtain a correction factor for the PM2.5 mass concentration of the DRX aerosol monitor.

Table C-1.

Average Results for Sampling Runs to Calibrate Mass Concentration of DRX to PMIs (µg/m^3)		
Aerosol Sampler	**Sampling Run 1**	**Sampling Run 2**
PMI's	18.305	11.074
DRX	21.542	12.230

DRX Correction Factor = [(18.305/21.542) + (11.074/12.230)]/2 = 0.877

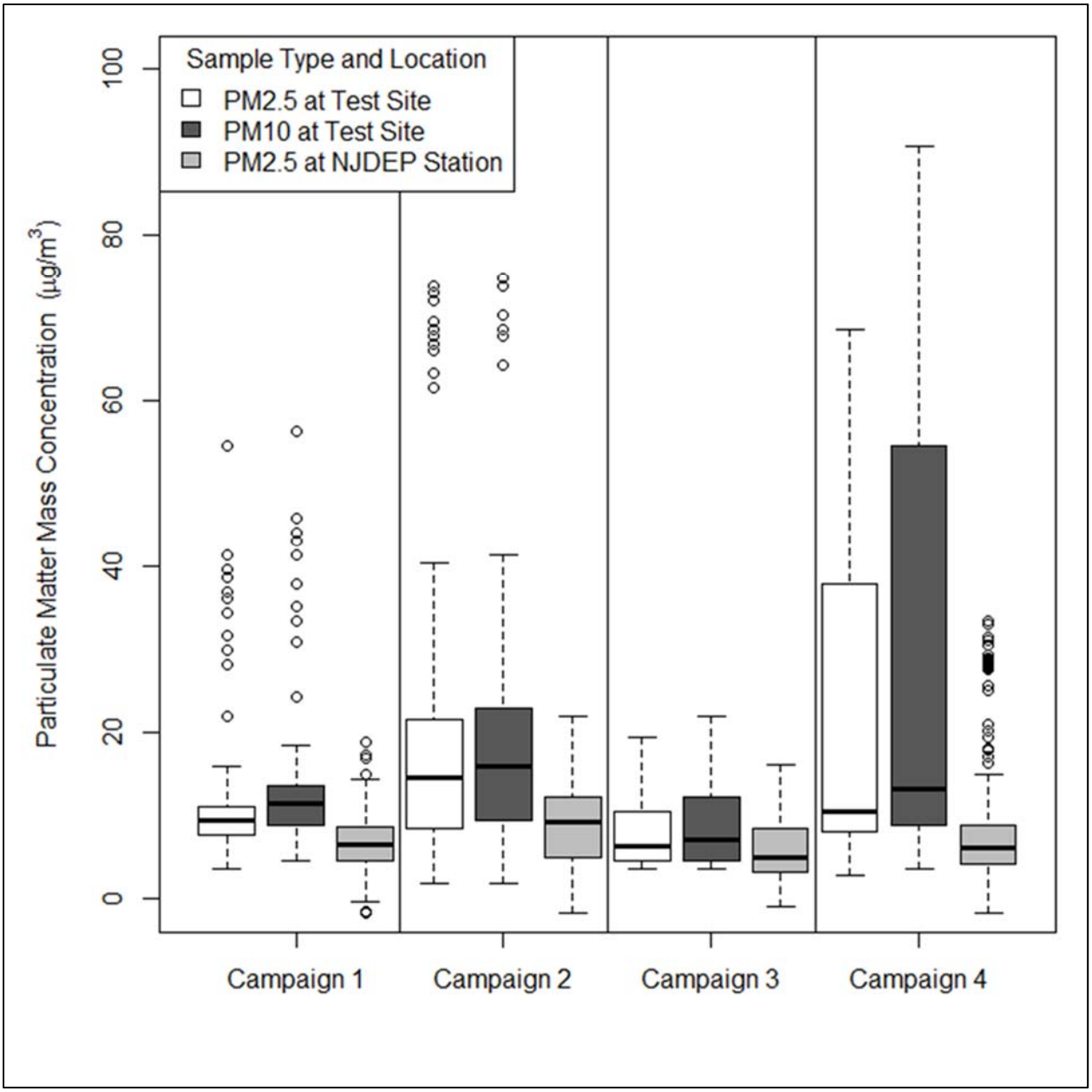

Figure C-1.

PM2.5 and PM10 mass concentration recorded daily for one hour at the sampling site using the DRX (TSI Inc.) versus PM2.5 mass concentration recorded hourly at a nearby NJDEP station by beta attenuation monitor.

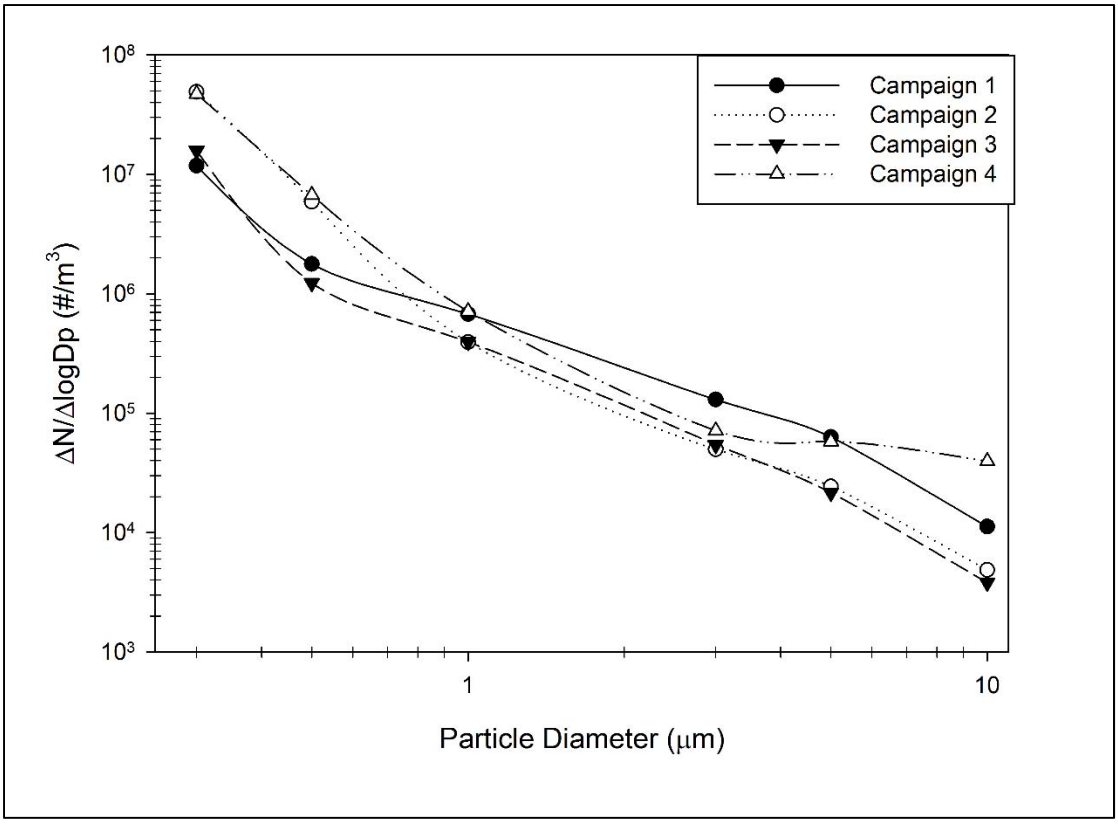

Figure C-2.

Normalized particle number concentrations gathered daily by the optical particle counter (Aerotrak handheld particle counter 9306, TSI Inc.) averaged across each sampling campaign.

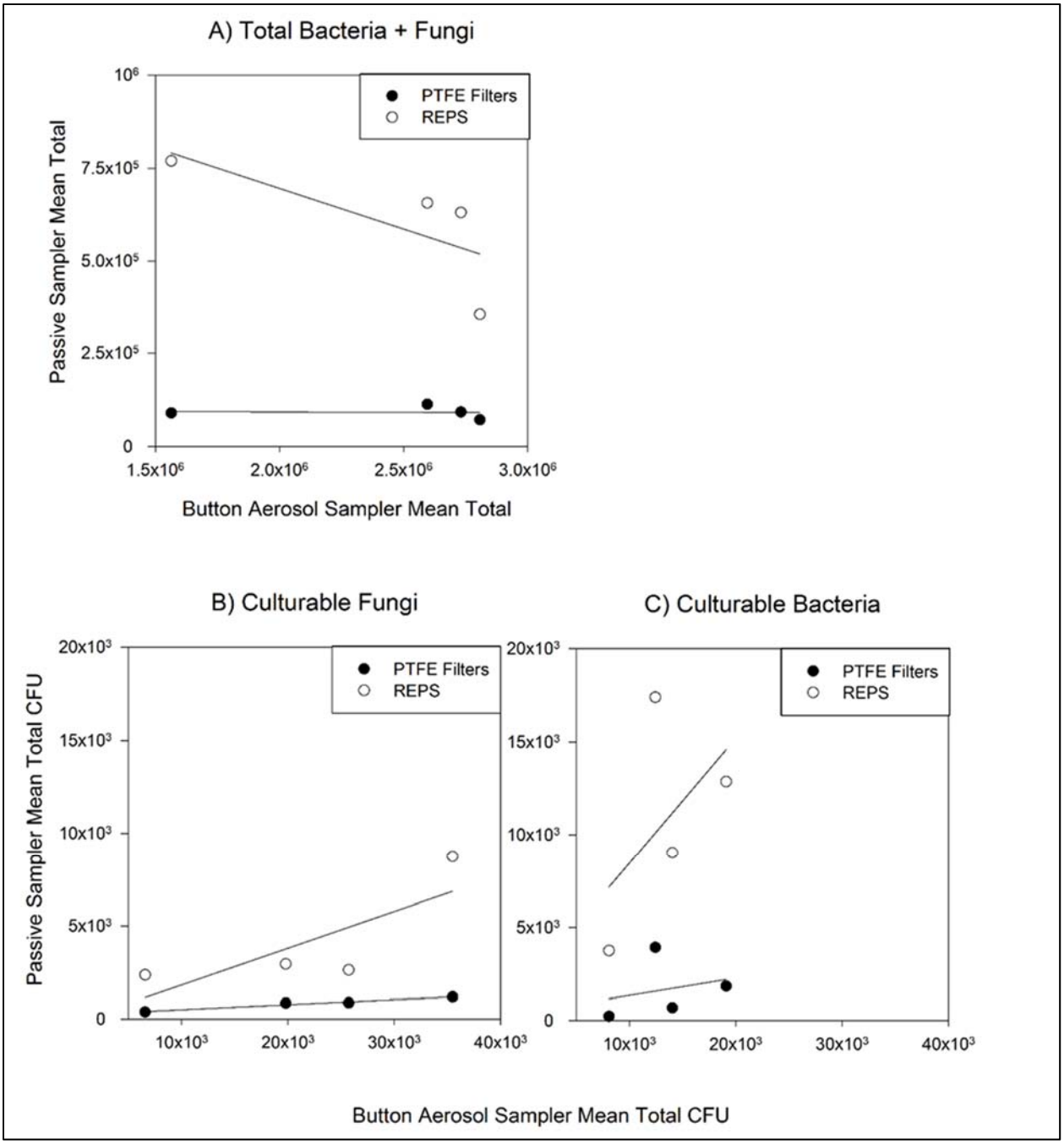

Figure C-3.

Correlation between bioaerosols collected by Button Aerosol Samplers (active reference) and passive samplers for total bacteria and fungi (A), total culturable bacteria (B), and total culturable fungi (C).

Appendix C2. References

TSI Incorporated: http://www.tsi.com/uploadedFiles/_Site_Root/Products/Literature/Application_Notes/EXPMN-005_DRX-Calibration-A4.pdf, last access: May 20, 2016, 2012.