Literature Review of Real-time Pathogen Detection in Urban Environments

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***Abstract*—Biosensors can be an extremely useful tool for detecting and responding to pathogens. There are a number of biosensor relevant technologies in existence for the real-time detection of pathogens. Urban environments add greater complexity to the sensor system. Some existing solutions and future challenges are discussed.**

***Index Terms*—biosensor, pathogen detection, real-time**

I. Introduction

THE importance of biosensors and their development in detecting and identifying biological pathogens has grown with the threat of biological warfare and the impending danger of epidemics. Biological pathogens are agents, commonly bacteria and viruses, known to cause disease in living organisms such as plants, animals, and humans.

Bacterial agents are single-celled organisms that cause illness in humans by either infecting tissue or releasing biotoxins [1]. A virus is a small microorganism consisting of genetic material, antigenic protein coat, and sometimes a lipoprotein envelope. Virus cannot perform independent metabolic activity and thus requires a host to provide energy and raw materials for the virus' own replication and functioning.

The most pressing need regarding biological pathogens is preventing and containing outbreaks. In the United States, the most recognized outbreaks have been foodborne illness caused by the bacteria *Escherichia coli*, *campylobacter*, and *salmonella*. Recently, the bacterium *Serratia marcescens* has been popping up in hospitals across the United States and caused deaths of patients. Viral outbreaks also occur; the most notable viruses that are cause for concern include influenza viruses, SARS coronavirus, *Flavivirus*, and viral meningitis.

Surveying and tracking outbreaks is a complicated task for public health departments and epidemiologists. The primary goal of the investigation is finding the origin of the outbreak. This assists in tracking the spread of the outbreak and identifying other people who may have come in contact with the pathogen. With this information, it is possible to focus geographical areas and populations requiring testing and treatment. These investigations are time consuming and costly. Traditional methods in identifying specific bacterial strains, i.e. plating and culture, are labor intensive manual processes, revealing a dire need for a device that provides real-time detection and identification.

Bacterial and viral pathogens are ideal for weaponization. Bacterial agents that are likely to be (or already have been) weaponized are *Mycobacterium tuberculosis*, *Yersinia pestis*, *Bacillus anthracis*, *Francisella tularensis* [1]. Weaponizable viral agents are primarily marburg virus, smallpox, venezuelan equine encephalitis, yellow fever, and ebola. These pathogens are optimal for aerosol delivery because of the microorganisms' stability and potency retention.

II. Existing Approaches

Traditional methods for detecting and identifying pathogens in various samples start with the separation of the pathogen from a sample – food, water, soil, or blood – and then culture enhancement to increase the number of the target pathogen. Next, an isolated colony of the target is examined under the microscope for physiological characteristics. Additional biochemical and metabolic indicators assist in identification. This technique is time consuming, taking up to ten days to confirm the identity of a pathogen [2]. More modern identification methods utilize tools that are based on molecular biology, such as Polymerase Chain Reaction (PCR, genetic technique) and Enzyme-Linked Immunosorbant Assay (ELISA, immunological technique). The molecular techniques decrease the amount of time testing requires as well as increase the specificity and sensitivity of the results. However, these assays tend to require a vast amount of sampling and purification [3].

Biosensors have a diverse range of applications. Specific properties of biosensors, or a system thereof, vary with the end user and their intentions. Many of the highest intensity efforts are focused on a national biodefense program, preparing tools to aid with "threat awareness, prevention and protection, surveillance and detection, post-attack response and recovery" in the event of biological weapon attacks [4]. The National Research Council has also supported work in the vein of counter-terrorism. They emphasize smallpox since it is

Manuscript submitted 10 May, 2011.

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considered an ideal biological warfare agent [5]. The Departments of Homeland Security and Energy have joined forces in the SERRI project to develop biosensors [6], [7]. DHS is also supporting industry efforts, such as the Luminex xMAP, to develop "fully automated" biosensors for early warning of airborne agents [8].

Biosensors have increased the speed and accuracy of pathogen detection, and provide methods that need little or no sample preparation. However, real-time detection is still in the works. The development of a biosensor that can provide a real-time analysis of the environment will assist in the quick containment of outbreaks and biological attacks by sorting out the infected from the non-infected and preventing a wide-spread illness and associated chaos. The ability to identify the agent quickly will also help emergency workers and emergency preparedness programs to act in the correct method and provide appropriate medicine or treatment to the infected people and environment.

Lab-on-a-chip biosensors have also garnered interest. Applications range from soldiers in hostile environment [4] to border monitoring at ports, airports and other border crossings. As these sensor systems mature, it is likely they will percolate into greater use in other areas, such as epidemic management, outbreak or microbial source tracking and hospital monitoring for the World Health Organization [9] or for emergency response, such as Community Emergency Response Teams [1]. Even more industry and consumer driven uses are likely to emerge as costs become less prohibitive. Food industries can detect contamination by bacteria and protozoa [10],[11]. Water treatment and other infrastructure areas can also be monitored for contaminants or infection by groups like the Environmental Protection Agency [9]-[11].

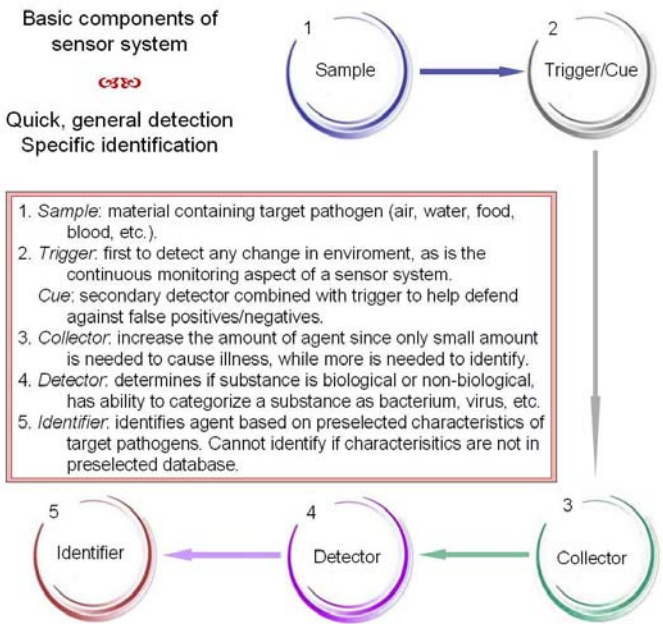

Figure 1: The basic components contained in a sensor system

III. Overview of Technologies

Biosensor is a broad term for a device that can detect substances that are biological or chemical in nature using various biochemical reactions, enzymes, whole cells, tissues and antibodies. The biosensor converts the data to a readable format, usually through an electrical, thermal or optical signal. Sensing approaches include point detection, standoff technologies, immunoassay, and nucleic acid sequence identification. Point detection requires the biological agent to be introduced directly to the sensor and utilizes trigger/cue, sampler/collector and identifier in the system [1]. Standoff technologies are able to function at a distance from the source. Standoff technology does not use the same components as the point detection system, often utilizing light sources instead, such as a laser [1]. Immunoassays use antibodies to target specified antigens, resulting in an antibody-antigen complex. DNA is also a target for biosensor technologies, focusing on nucleic acid sequence to identify a living organism (bacteria, viruses, fungus).

Current technology has enabled researchers to utilize diagnostic assays in the identification of biomaterials in a given sample. These techniques utilize probes and assays to categorize targets. One method involves the selection of known genome strands to identify groups of known pathogens like the 16S rRNA gene, virulence genes, and antibiotic resistance genes. All of these genes have been incorporated for microarray probes in order to identify on the species level [12]. Microarrays allow users to get faster, more sensitive results, when compared to traditional methods that require growing pathogens in culture. While this method is feasible, the cost of the end products have impeded their use in mainstream research [13]. There are also some specific methods that identify the whole genome of the biomaterial.

Microfabrication technology has enabled the development of microfluidic devices. They are normally made up of channels for liquids and small sensing chambers [10]. This design allows for a high surface to volume ratio, which allows for much faster analysis [10]. Microfluidic technology is integral to the idea of Lab-on-a-Chip technology. The samples used in microfluidics can be both liquids and gases, with the common property that they can both be deformed with relative ease [14]. The scaling of whole laboratories from ten meters to the scale of micrometers has allowed the "dramatic reduction of the amount of required sample" [14].

Optical sensors have made several technological advances in research on pathogen detection. The sensors can detect flouresence, surface plasmon resonance, and piezoelectric changes.

Another method of detection is through bioreporters. This uses living organisms as detectors in an environments and measures “cellular signal outputs” to identify changes as they take place. This has been used in water quality monitoring and “specific identification” of certain organisms that are threatening to human health [9] as well as plants identifying molecules associated with explosive materials by rapidly changing color through synthetic signal transduction pathways [15]. Eukaryotic cells are particularly accurate indicators of human risk [9]. These systems take advantage of the rapid response times innate to cellular signaling and organisms responding to their environment, and make short analysis time and real to near real-time measurement feasible [9]. Bioreporters are also easy to use, and may be more portable and economical than many currently available systems [9]. However, because these systems are actually living organisms, they must “remain viable” in the environment or “testing regimen” where they are being exposed. This requires the care and maintenance appropriate to the selected bioreporter species [9].

E-noses were developed as a novel method of rapidly detecting biological agents in a median. The idea was inspired by the natural phenomenon of noses that were adapted to pick molecules in the air. For example, a human nose is capable picking up molecules which are then decoded by the brain to be representative of particular matter. Molecules in the air have a specific chemical makeup that will respond to specific receptors located in the noses passage ways. Depending on which receptor responds and the amount of them, we can perceive a certain smell and which intensity is currently present. “A gas sensor array consisting of six metal oxide gas sensors and one electrochemical gas sensor” developed to detect pathogens that may exist in the wound of a patient [16].

Iowa State is currently developing their version of the e-nose to detect pathogens that may exist in the manufacturing process of food products. Their version of the e-nose was built using an array of semiconductors that would be able to map the chemical composition of the test sample. The resulting map would then be compared to a previously stored library of chemical chains [17]. “Aroma pattern for fresh ground beef and one for 1-, 2-, and 3-day old spoiled beef can be stored and named” [17].

Current goals involved in the development of e-noses are building a universal library to compare results against and the development of “a standard curve of some potential volatile compounds that can be used to develop some specific substrate” to detect other compounds of interest [17].

A wide variety of other techniques have also been employed to develop pathogen detection. Electrochemical techniques are logical extensions of traditional PCR style techniques and have been used for DNA sensors and immunosensors in water quality, food-borne microorganisms and could be extended to urban environment detection [9] (Pedrero). Electromechanical and chemical sensing approaches have also been utilized in similar settings [9].

Nanomaterials have begun to move into many technological regimes, and biosensors are no different. Coupled with other technologies, nanomaterials can expand the functionality and applicability of many existing techniques [11]. Combining optics with plasmonic nanohole material has been used with specific antibodies to accurately identify “intact viruses” from “biological media”. This approach is label-free, requires little processing of samples, and has promising sensitivity [18]. By bringing sensor components into the nanoscale, both detection limit and sensitivity are improved as the pathogens being detected are closer in scale to the equipment being used [11]. The increase in surface area possible with nanofeature material also makes the possible device sensitivity much higher [11].

IV. Existing Platforms

BioWatch Program:

Managed and funded by the Department of Homeland Security (DHS), an interagency program known as the BioWatch Program works alongside the Environmental Protection Agency (EPA) and the Centers for Disease Control (CDC) to employ a string of pathogen detectors. This project was deployed as the first of its kind, an early warning system to detect a biological attack. The sensors, maintained by the EPA, are mounted at preexisting air quality monitoring stations throughout approximately thirty cities. The sensors collect particles in the air which pass through filters, which are collected manually and regularly. The equipment used in the program is based primarily on a system known as BASIS, the Biological Aerosol Sentry and Information System. Specifically, the design of the filter mechanism can determine if an attack is taking place by automatic sequential filtering. The BASIS system was deployed in 2002 for indoor and outdoor monitoring at the Olympics and it was there that is was tested for an urban environment. Only a few of the results from these tests were released, it was shown to have high specificity and sensitivity while having less than 0.005% false positives, and although the capability of the sensor is promising, the system is labor intensive. These filters are to be collected and then transported to nearby laboratories for specific analysis coordinated by the CDC. The laboratory testing is carried out by state or local public health departments, the Federal Bureau of Investigation (FBI) acts as the lead agency that coordinates law enforcement response if a bioterrorism attack is confirmed [19].

In October 2003 in Texas, the first positive result was reported by the BioWatch Program. Tularemia was detected at low levels, and although the detection was modest, precautionary measures were taken by the authorities and

public health departments in regards to increasing surveillance, sampling, testing and assessing the environment to determine of what may have triggered the alert, such as a biological attack or natural conditions of the agent in the environment or if it just recently became present in the environment. It was concluded that it was not a result of a biological attack, however investigations were on-going and authorities chose to increase surveillance in the possible affected population. Without a sensor system, the detection of a bioterrorism attack and alteration in the natural environment (possible outbreak) would most likely occur though diagnosis once the illness already took hold and spread from the initial contaminated area [19].

TB Breathalyzer:

Rapid Biosensor Systems developed a breathalyzer that analyzes the sample via a displacement assay utilizing the evanescent wave and bio-optical sensing technologies for detecting tuberculosis. After successful clinical trials, the RBS TB Breathalyser is now undergoing a final test before being sent into production and provides a portable, non-invasive test that provides near real-time results. Additionally, the product does not require a clean room to be assembled nor does it necessarily require medical personnel to operate the sensor [20].

The product itself uses a single-use disposable collection tube in which the patient coughs into as well as a multi-use reader. The bottom of the collection tube is coated with a fluorescent, biochemical analogue that specifically reacts to Mycobacterium tuberculosis. The tube is easily sealed by a simple push/twist movement which allows the sample to fall onto the sensor portion of the device, followed by its insertion into the reader. It is here in the reader the the sample undergoes a displacement assay using the evanescent wave technology. When the antigen, having higher bond strength to the antibodies for TB, displaces the fluorescent analogues, the diode laser detects a signal change due to a reduction in the signal after excitation, leading the unit to declare a positive result. All of this takes about two minutes for the entire testing process, and is followed by the destruction of the collection tube. The RBS Breathalyser has shown to have extremely high specificity and sensitivity, and, importantly, not compromised by the presence of other pathogens. HIV is a strong masking agent for TB and there tends to be a co-existence of the two disease in third world countries [20].

V. Future Challenges

The existing technology has a number of limitations that must be addressed before real-time pathogen detection is feasible in a wide-scale application. Based on an urban environment concept of operations, a system needs to be first and foremost set up for reliable field use. Urban environments are often dirty, both with biological, pollutant and inorganic materials, causing a lot of noise in detection (SRC). This makes sampling and sensitivity critical challenges, especially coupled with the short timelines necessary between sampling and reporting. Reliability is also a factor: results must be both accurate, consistent and without false negatives or positives and clearly indicated when multiple threats are being detected. Cost and deployability are the final hurdles in making it possible to effectively deploy sensors as an early warning system.

Environmental challenges vary with the type of sensor. Any sensor must function in its ambient environment, whether it is controlled, or must maintain accurate functioning in a range of temperature, humidity, weather, etc conditions [1]. Bioreporters are living, and must “remain viable” in any “environmental testing regimen” in which they are being utilized [9]. Label free detection also comes into play here. The field environment is not conducive to multiple steps of preparation and processing, making it critical that sensors be deployable and easy to utilize and read [9].

In addressing performance, multisensor fusion is a highly desirable trait [4]. A sensor that can detect only one threat, or possible even one variation of a threat is vulnerable to the remainder of the spectrum of possible pathogens. Wide spectrum detection is not enough, however. The selectivity of the sensor must be appropriate, and not set off alarms for threats that aren’t relevant [1]. Bioreporters in eukaryotic cells are often good indicators of human risk [9]. The sensitivity and sampling of the sensor impact efficacy, as low threshold limits and straightforward sampling technologies are enabling traits for early warning systems.

Finally, the interface with users must be appropriately addressed. The systems must be cost effective to deploy, preferably reusable or with significant device longevity, and report results in real or near real-time to be useful in an urban threat environment [9]. The results must be reliable: the devices must be trusted not to raise costly and frightening false alarms, without neglecting to alert users to possible threats when it is still early enough for action to be taken. Optimizing this balance will vary with users and concepts of operations, but will always be critical.

Given the threats faced, and the quality of life improvements that can be extended from a multisensor real-time pathogen detector, it makes it an area of extremely valuable interest. The challenges to be overcome are significant, but that makes success that much more rewarding.

References

[1] National Institute of Justice, “An Introduction to Biological Agent Detection Equipment for Emergency First Responders,” in *NIJ Guide*, 101-00.

[2] P. Feng. *Rapid Methods for Detecting Foodborne Pathogens*. Bacteriological Analytical Manual, 8th Edition, January 2001.

[3] M. Zourob, et al. ed. *Principles of Bacterial Detection: Biosensors, Recognition Receptors and Microsystems.* Springer, New York, NY, 2008.
[4] Plamen A. Demirev, Andrew B. Feldman, and Jeffrey S. Lin. *Chemical and Biological Weapons: Current Concepts for Future Defenses.* Johns Hopkins APL Technical Digest, Volume 26, Number 4. 2005.
[5] K. Donaldson, et al. *A rapid detection method for Vaccinia virus, the surrogate for smallpox virus.*
[6] A. Friedli. "Phase 1 Final Report," *SERRI Project:Biosensor Research.* Supported by Dept Homeland Security and Dept. of Energy Interagency Agreement.
[7] C.J. Bruckner-Lea. *Biosensor Systems for Homeland Security.*
[8] *Homeland Security Newswire.*
[9] K. Sengupta. *Environmental Microbiology: Current Technology and Water Applications*, 2011.
[10] H. Jinseok, et al. *An Overview of Recent Strategies in Pathogen Sensing.*
[11] S. Xu, R. Mutharasan. "The Coming Together of the Sciences: Biosensors for the Detection of Waterborne Pathogens Using Antibodeies and Gene-based Recognition Chemistries."
[12] R.V. Satya, N. Zavaljevski, K. Kumar and J. Reifman. "A high-throughput pipeline for designing mircroarray-based pathogen diagnostic assays," *BMC Bioinformatics* 2008, 9:185. Published 10 April 2008.
[13] G.J. Vora, C.E. Meador, D.S. Stenger, J.D. Andreadis. "Nucleic Acid Amplification Strategies for DNA Microarray-Based Pathogen Detection."
[14] H. Bruss. *Microfluidics.* Oxford: Oxford Univ. Pr., 2010.
[15] M.S. Antunes, K.J. Morey, J.J. Smith, K.D. Albrecht, T.A. Bowen, et al. "Programmable Ligand Detection System in Plants through a Synthetic Signal Transduction Pathway." PLoS ONE 6(1): e16292. doi:10.1371/journal.pone.0016292. 2011.
[16] F. Tian, X. Xu, Y. Shen, J. Yan, Q. He, J. Ma, T. Liu, *Detection of Wound Pathogen by an Intellegent Electronic Nose. Sensors and Materials*. MYU Tokyo, 2009.
[17] A. Pometto, *Electronic Nose for Rapid Detection of Food Borne Pathogens in Meats*. Iowa State University.
[18] A.A. Yanik, M. Huang, O. Kamohara, A. Artar, T.W. Geisbert, J.H. Connor. "An Optofluidic Nanoplasmonic Biosensor for Direct Detection of Live Viruses from Biological Media," *Altug Nano Letters* 2010 *10* (12), 4962-4969.
[19] D.A. Shea, S.A. Lister. "The BioWatch Program: Detection of Bioterrorism". Congressional Research Service Report No. RL 32152. November 19, 2003.
[20] D. Camilleri. "New Screening Solution Offers Hope in the Battle Against TB". *Rapid Biosensor Systems*, 2008.