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Detection of Environmental Toxins in Mixed Matrices of Tap Water, Soil, Food Waste, Serum and Milk Using Hememics Biosensor

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Abstract

Exposure to toxins can lead to a wide range of adverse health effects, including respiratory problems, neurological disorders, cancer, and reproductive issues. Toxins can come from various sources, such as industrial waste, agricultural runoff, and household chemicals. Therefore, detecting and monitoring toxins in the environment is crucial for protecting human health and the environment. This study aimed to evaluate the performance of Hememics biosensor system in detecting environmental toxins such as Ricin and Staphylococcal enterotoxin B (SEB) in mixed matrixes. When Ricin and SEB are spiked into soil, chopped lettuce, tap water, milk and serum, the biosensor was able to detect these toxins, without sample processing, at a level of detection comparable to lab testing with high sensitivity and specificity. Furthermore, Hememics biosensor system is designed to be network-enabled, which means that results can be transmitted to relevant agencies for quick decisions. This feature is crucial in cases where quick action is needed to prevent further contamination or exposure to harmful toxins.

Keywords

Portable Biosensor, Graphene Based Biochip, HemChip™, Rapid Detection, Field Use, Networking

1. Introduction

Testing samples collected in the field for various analytes can be a challenging task, especially when dealing with dirty samples [1] [2]. Often, these samples need

to be transported to a central lab for testing, which can be both time-consuming and expensive. Environmental testing in the lab typically involves collecting samples from the environment and bringing them back to the lab for processing and analysis. This can involve complex sample preparation techniques to extract the target analytes from the sample matrix, followed by analysis using sophisticated analytical instruments. Depending on the type of analysis, this process can take anywhere from a few hours to several days or even weeks to complete. In addition, there is a risk of contamination during the sample collection, transport, and processing steps, which can lead to inaccurate results [3] [4]. Other more direct traditional methods for testing samples in the field involved using relatively simple, manual techniques that are not always very accurate or reliable [5] [6]. For example, one common method was to visually inspect the sample for signs of contamination or disease. Another approach involved culturing the sample in a laboratory dish and observing any growth. New technologies and methods have been developed in recent years to address these challenges and improve the accuracy and reliability of field testing [7]. For example, lateral flow assays, microfluidic devices, and portable PCR machines [8] [9] [10]. Conventional lateral flow assays, which are commonly used for point-of-care testing, often fail when it comes to testing samples from the field due to the presence of various contaminants [11]. In particular, the presence of mud, dirt, and other impurities in samples collected from barns, animal pens, or other outdoor locations can easily clot the paper-based strips used in these assays, leading to inaccurate or inconclusive results. Microfluidic devices are also small and portable, but they use channels and chambers to manipulate and analyze fluids [9]. Portable PCR machines use a thermal cycling process to amplify DNA, allowing for highly specific detection of pathogens. These methods are time-consuming and often require specialized equipment and expertise. In addition, they are not always able to detect all types of contaminants or diseases, and they are prone to error and false positives [12].

There is a pressing need for innovative solutions that can address these challenges and provide accurate and efficient testing in the field [13] [14]. In recent years, biosensors have emerged as a promising tool for environmental testing due to their ability to provide rapid and accurate results in the field [15]. Hememics, has developed a graphene-based biosensor platform that is well-suited for point-of-care testing in environmental settings. The Hememics biosensor system offers a distinct advantage over traditional methods, as it has the capability to simultaneously detect multiple molecular and antigen targets directly in the field. This feature makes it an attractive tool for environmental monitoring, as it allows for the direct detection of pathogens without the need for time-consuming and expensive sample preparation steps.

The objective of this study is to assess the ability of the Hememics biosensor system to detect Staphylococcal enterotoxin B (SEB) and ricin in complex matrices, including mud, serum, vegetable wash, and milk. This capability is a sig-

nificant advancement in the field of environmental monitoring, as traditional laboratory-based methods often require time-consuming and expensive sample preparation and analysis. With the Hememics biosensor, rapid and accurate detection of environmental toxins can be achieved on-site, providing valuable information for public health and safety.

2. Materials and Methods

2.1. The Biosensor System

The Hememics biosensor system consisted of two components manufactured by Hememics Biotechnologies (Gaithersburg, MD): a networking-enabled HemBox™ biosensor reader and a disposable HemChip™ for sample analysis. The HemBox™ is capable of networking, while the HemChip™ is equipped with a 32-plex biosensor array programmed with bioreceptors that can detect multiple analytes at once.

2.2. Preparation of HemChip™

To provide an adhesive surface to anchor amino aptamers, HemChip™ was washed with ethanol followed by DI water, bake at 70˚C for 15 min. Then, incubated for 15 minutes with 40 µl of the 1 mM pyrenebutyric acid succinimidyl ester (PBASE) in DMSO (Sigma, St. Louis, MO). HemChip™ was washed with DMSO and 1X PBS to remove the unbound PBASE.

2.3. Functionalization of HemChip™

To program the HemChip™ with specific bioreceptors, 200 - 400 pL of amino aptamer at the concentration of 100 mM was added onto the HemChip™ through a robotic guided micro dispensing process (Scienion, Germany) directly onto the sensor areas and incubated in a 60% humidity chamber at 40˚C for an hour. Bio-receptors were conjugated covalently to PBASE via amine ester reaction. Non-specific sites were blocked with a proprietary mixture of PEG/Branched PEG. Amine modified Aptamers against Ricin and SEB were obtained from IDT (Coralville, Iowa). At the end of the process, the unbound aptamers were aspirated off and then, the HemChip™ was dried stabilized in a proprietary process using Hemsol [16] [17] and freeze-dried overnight and then stored in vacuumed sealed pouch ready to be used (18 months shelf life).

2.4. Preparation of the Environmental Samples

A plastic disposable dispenser was designed with filter built into the nozzle to remove any large debris. The dispenser contained 1.0 mL of proprietary HemSol™ [17] lysis buffer. This solution was used to prepare several environmental samples: soil from our front yard (10% w/v), tap water (0.1 mL), 1% fat milk (0.1 mL), chopped lettuce (10% w/v), and Fetal Bovine Serum (0.1 mL). These samples were mixed directly into the HemSol™ lysis buffer. Staphylococcus enterotoxin B (SEB) from Millipore Sigma (St. Louis, MO) and Ricin from Antibo-

dies-online (Limerick, PA) were purchased and added to these samples at a concentration of 0.1 μM. Without any further processing, a small amount of each environmental sample (~50 μl) was placed directly onto the HemChip™ for testing.

2.5. Preparation of the Environmental Samples

Statistical analysis was performed using GraphPad Prism software to determine the sensitivity, specificity, and limit of detection of the Hememics biosensor system. The sensitivity of the biosensor was determined as the ability to detect the presence of a target molecule. Specificity was determined as the ability to detect only the target molecule and not other molecules. The limit of detection was determined as the lowest concentration of the target molecule that could be detected by the biosensor.

3. Results

Illustration of the Mechanism of HemChip™ Sensor: The Hememics biosensor system consisted of two components manufactured by Hememics Biotechnologies (Gaithersburg, MD) (Figure 1): a network enable HemBox™ and the heart of the system, the HemChip™ (Figure 1).

The HemChip™ was designed with an array of thirty-two (32) independent sensors that could identify multiple targets spontaneously. The first step was to stabilize aptamers specifically designed for a specific target on an individual HemChip™. Once the aptamers were stabilized and preserved on the HemChip™, it was inserted into the HemBox™, which read and interpreted the baseline electrical resistance. A fluid sample was then introduced to the HemChip™ and

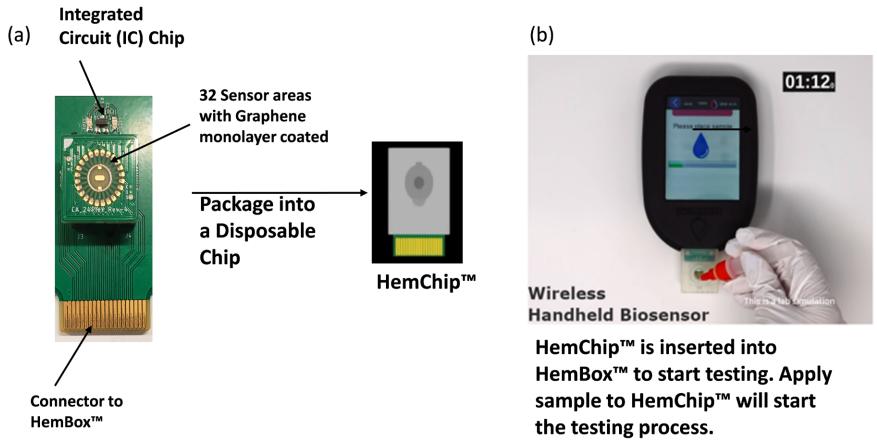

Figure 1. The anatomy of a Hememics Biosensor System. A disposable HemChip™ contains 32 sensor areas coated with graphene monolayer. Each chip contains an IC to identify chip type, location of specific bioreceptor on the sensor areas, expiration, etc. The HemChip™ is connected to the HemBox™. In a one-step process, a sample is applied to the receptible port on the chip, using a plastic disposable dispenser, for testing and analysis. Results are visible in less than 5 minutes or can be transmitted wirelessly or to a cell phone via Bluetooth.

within 5 minutes, the HemBox™ could detect if there was an interruption in the electric current, indicating a binding effect between the aptamers and its target (Figure 2). Based on the presence or absence of a response, the HemBox™ delivered either a detected or not detected readout.

To evaluate the ability of HemChip™, which had been programmed with Ricin and SEB aptamers, to detect environmental toxins in tap water and soil, experiments were conducted. To carry out the experiments, tap water and soil were spiked with Staphylococcal enterotoxin B (SEB) and Ricin. The spiked samples were then mixed with the provided buffer and carefully placed onto the chip. Several concentrations of the samples were tested, and electrical impedance was recorded as evidence of binding (Figure 3). The change in electrical impedance was compared to the negative controls, which included buffer alone and buffer spiked with BSA (bovine serum albumin). The data demonstrated a dose-response relationship in binding, as indicated by the corresponding change in electrical impedance. This suggests that the binding is specific and dose-dependent. In contrast, the negative controls showed a baseline electrical signal, indicating the absence of any specific binding.

Additional experiments were conducted using HemChip™ technology to determine the lowest detection limit (LOD) for SEB and Ricin in various environmental matrices such as tap water, soil, serum, chopped lettuce, and 1% fat milk. HemChip™ was pre-programmed with aptamers specific to SEB and Ricin, and samples from each matrix were introduced into the chip. The minimum concentration of SEB and Ricin that could be detected was recorded and tabulated in Table 1. The values in Table 1 provide information on the sensitivity of the HemChip™ technology in detecting SEB and Ricin in different environmental matrices.

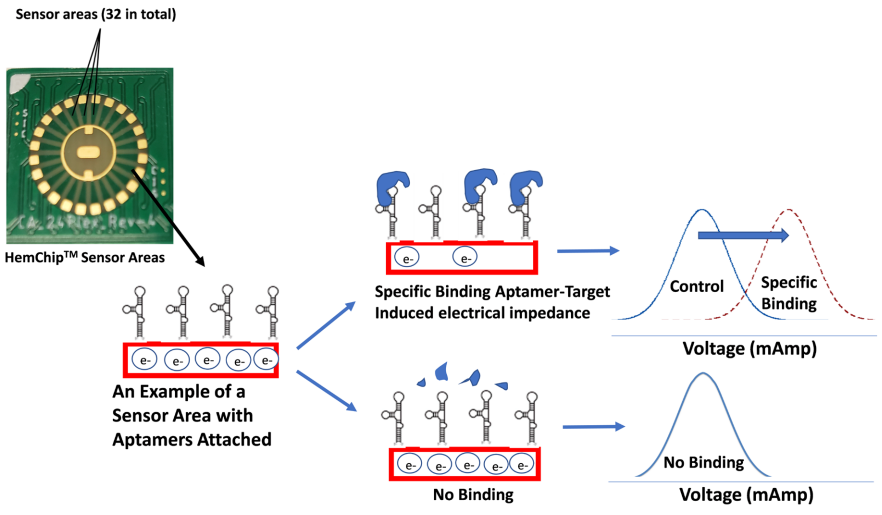

Figure 2. Illustration of HemChip™ sensor mechanism. Aptamers were attached to the sensor areas. If there was a specific binding interaction, there will be an electrical impedance resulting in a shift in voltage across the sensor area. Similarly, no electrical output changes occurred if there was no binding event.

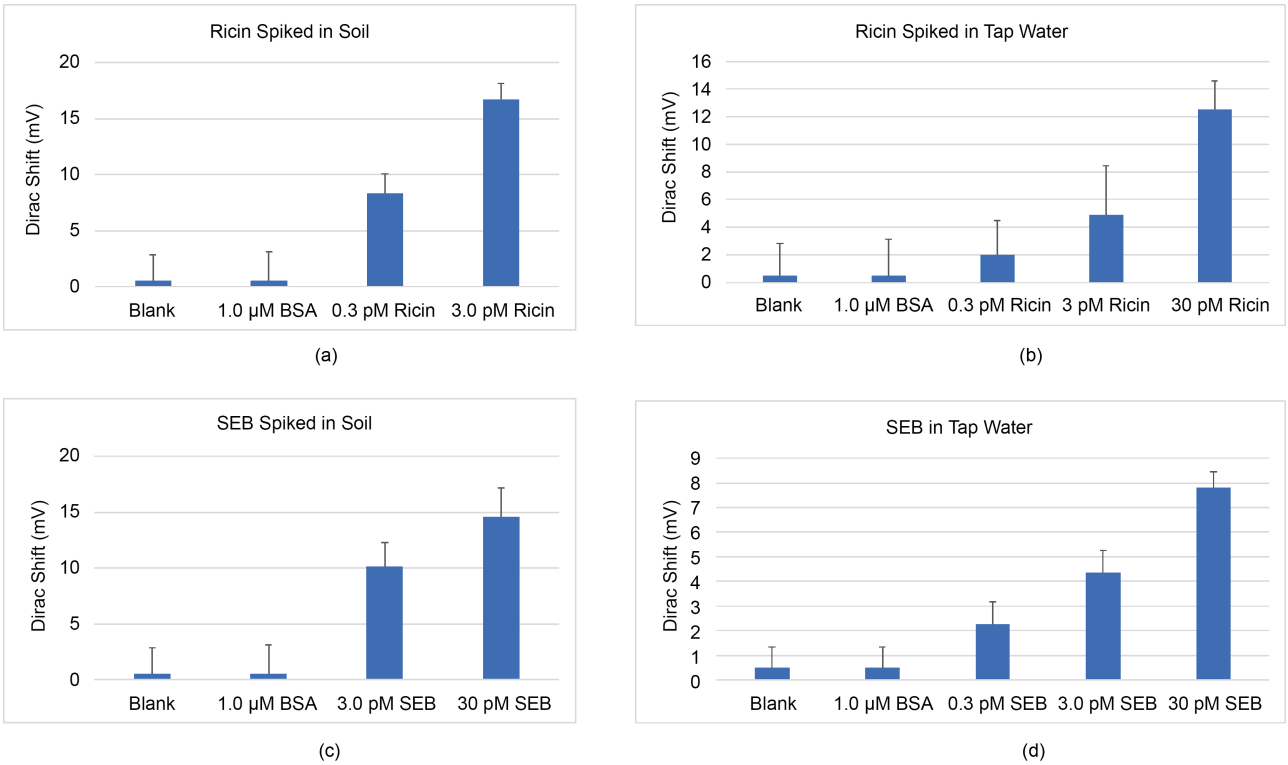

Figure 3. Specific detection of SEB and Ricin using HemChip™. The samples of Ricin in soil (A) and tap water (B), as well as SEB in soil (C) and tap water (D), were tested for their binding to the HemChip™ that was programmed with aptamers specifically targeting Ricin (panels A&B) and SEB (panels C&D). The direct shift, representing the electrical impedance, was recorded and plotted against various concentrations of these targets. In the experiments, the negative controls included the blank control, which was the buffer alone, and buffer spiked with BSA (bovine serum albumin). Each data point represents the average and standard deviation of 10 data points.

Table 1. Lowest detection limits (LOD) for SEB and Ricin in various environmental matrices using Hememics biosensor technology

Sample	Matrix	LoD
Ricin	PBS	3.0 pM
	Soil	0.03 pM
	Chopped Lettuce	30.0 pM
	Tap Water	3.0 pM
SEB	PBS	4.0 pM
	Soil	0.04 pM
	Milk	4.0 pM
	Tap Water	4.0 pM
	Serum	40 pM

4. Discussion

Previous studies on Ricin detection have reported various levels of detection using different techniques. For instance, SPR detected Ricin at a level of 0.5 ng/mL [18], while MALDI-TOF MS detected it at 50 ng/mL [19]. The most sensitive

detection was achieved by immunoaffinity and liquid chromatography-tandem mass spectrometry, which had a limit of detection of 0.1 ng/mL (1.56 pM) [20]. For SEB detection using Nanowire field effect transistors (nano-FET), a detection limit was reported at 0.01 - 0.035 pM [21] while other techniques reported a detection limit of 0.1 ng/mL (about 4 pM) [22] to 4 ng/mL (about 160 pM) [23]. In contrast, our study using the HemChip™ biosensor system detected Ricin and SEB at much lower levels, specifically 0.03 pM and 0.04 pM in soil samples, respectively. Taken all of these together, the Hememics biosensor system has shown superior performance in detecting toxins like SEB and Ricin. This represents a significant advancement in the field of environmental testing, as it allows for direct testing of samples from environmental matrices without the need for processing. While further work is needed to refine and validate the technology, the potential for the Hememics biosensor system to be a valuable tool for field testing and environmental monitoring is promising, and has not been possible before.

The heart of the system is the HemChip™, which contains 32-plex circuits that can be coated with multiple aptamers to detect multiple toxins in a single sample. The HemChip™ utilizes GFET technology, which is a new and emerging technology that offers several advantages over traditional biosensors [24] [25]. GFETs can detect target molecules in less than 5 minutes, making them ideal for rapid detection applications. They are also highly sensitive and selective, making them ideal for detecting low levels of toxins in complex matrices [26]. Unlike traditional biosensors that use microfluidics, the interaction between the sample and the bioreceptor on the HemChip™ is static. This allows for the detection of target molecules in dirty or complex matrices, such as food or environmental samples.

Compared to traditional laboratory testing methods, which require samples to be shipped to a central lab for analysis, the Hememics biosensor system allowed for direct testing on site. This feature significantly reduces the time and cost associated with sample processing and transport, making it a valuable tool for environmental health and safety agencies.

Moreover, the networking capability of the HemBox™ enables real-time transmission of results to relevant agencies, allowing for quick and informed decision making. The ability to detect toxins on-site and in real-time provides a significant advantage over traditional laboratory methods, which can take days or even weeks to produce results.

Overall, the Hememics biosensor system holds great potential for environmental monitoring, particularly in the detection of environmental toxins. With further development and optimization, it could become a game-changer for the field of environmental health and safety.

5. Conclusion

The Hememics biosensor system demonstrated high sensitivity and selectivity in

detecting environmental toxins such as ricin and SEB in water, mud, serum, vegetable wash, and milk. The system provides a rapid, on-site detection method that eliminates the need for sample processing and transport to central laboratories, which can save time and resources. The ability of the system to detect toxins directly in the field allows for quick decision-making and appropriate actions to be taken to protect public health. The networking capability of the HemBox™ further enhances its usefulness in real-time monitoring and reporting of environmental toxins. Overall, this study highlights the potential of the Hememics biosensor system as a powerful tool for environmental monitoring and public health protection.

6. Declarations

Author contribution: Researchers David Huy Ho, Srivatsa Aithal and Nathan Ho, contributed the following: (a) research concept and design, (b) writing the article, (c) critical revision of the article, (d) final approval of the article. Researchers Sujasha Gupta, Khanh Duong, Ankit Kumar, Dong Dong Liu and John Warden contributed the following: (a) collection and/ or assembly of data, (b) data analysis and interpretation.

Ethics Approval

All authors have read, understood, and have complied as applicable with the statement on "ethical responsibilities of authors" as found in the instructions for authors and are aware that with minor exceptions, no changes can be made to authorship once the paper is submitted.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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