REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

A Carbon Nanotube-based Field-effect Transistor Biosensor For The Detection Of Fentanyl Exposure

Wenting Shao University of Pittsburgh **Alexander Star** University of Pittsburgh

Fentanyl is a potent synthetic opioid that is used as a pain reliever and as an anesthetic. It is approximately 50-100 times more potent than morphine. However, due to their pharmacological effects, the overdose of fentanyl can cause difficulties in breathing, and can lead to death.

According to Centers for Disease Control and Prevention (CDC), synthetic opioids are the primary driver of overdose deaths in the United States. From October 2020 to October 2021, more than 69,000 Americans died of drug overdoses related to synthetic opioids like fentanyl. At the same time, fentanyl can also be found in combination with heroin, counterfeit pills, and cocaine. Therefore, there is a great need of a highly sensitive, portable and inexpensive analytical technique that can quickly indicate the presence and relative threat of fentanyl.

In human body, fentanyl gets metabolized to norfentanyl via oxidative N-dealkylation and to 4-ANPP via hydrolysis. Norfentanyl, as the primary inactive metabolite of fentanyl, can be detected in body fluids with wider detection window. Our work aims to develop a functionalized carbon nanotube-based biosensor for ultrasensitive norfentanyl detection in body fluids such as urine, serum, and sweat and consequently making time-sensitive decisions.

Carbon nanotube-based field-effect transistors (NTFETs) have shown remarkable sensitivity and low detection limits for a variety of biological analytes. FET-based biosensors are dependent on the interactions of biomolecules with the semiconducting single-walled carbon nanotube (SWCNT) channel causing alteration in the dielectric environment, charge carrier density, work function modulation, or charge transfer into the nanotube. NTFETs can be functionalized with custom-designed selective chemistry to preferentially interact with desired biomolecules, demonstrating excellent sensing behavior in complex media like urine or plasma.

For norfentanyl sensing, norfentanyl antibody was functionalized on SWCNTs via two different approaches – direct coupling and through gold nanoparticles (AuNPs). When tested against a series of norfentanyl solutions in phosphate buffered saline (PBS), both types of biosensors exhibited sensing capabilities for norfentanyl, with limit of detection (LOD) estimated to be around 1 fg/mL and a response time within 5 min. Interestingly, the calibration plots for the two types of norfentanyl biosensors showed opposite trends. The different sensing behaviors are indicative of different sensing mechanisms for the two types of biosensors.

The norfentanyl sensing capability of the two types of sensors remained when tested in a more complex system, such as synthetic urine. However, a decrease in the sensing performance (sensitivity and LOD) was observed due to the interference of other non-specific components in the synthetic urine. When comparing biosensors employing direct coupling and AuNP approach, it was discovered that the AuNP decorated biosensors are less susceptible to nonspecific species present in synthetic urine.

In conclusion, the norfentanyl antibody-functionalized carbon nanotube-based FET biosensor has shown great potential for the development of a rapid, sensitive, portable and inexpensive device for detection of fentanyl exposure. We envision our norfentanyl biosensor can enhance the safety and security of both the first responders and the overdose victims.

This work was supported by the Chem-Bio Diagnostics program grant HDTRA1-21-1-0009 from the Department of Defense Chemical and Biological Defense program through the Defense Threat Reduction Agency (DTRA).