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

Electrochemical Aptamer-based Sensors: A Field-applicable Approach For Real-time Monitoring Of Small Molecule Targets And Beyond.

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Problem Scope: Responding to emerging threats in the field requires detection capabilities that provide a rapid read-out. However, many existing diagnostic technologies are either slow, nonspecific, or fail to provide meaningful information when employed in realistic human or environmental samples. To enhance the warfighters' ability to detect and respond to emerging threats, we require a field-ready, generalizable technology to measure concentrations of specific molecules in real time.

Electrochemical Aptamer-based Sensors Provide Rapid Measurements Directly in Complex Samples:
Electrochemical aptamer-based sensors offer a platform technology to achieve real-time molecular monitoring of a range of targets. To date, they are among the only diagnostic technologies to work directly in situ in the living body. These sensors consist of an aptamer (an artificially selected, nucleic acid receptor) modified with a redox reporter and attached to an electrode via a self-assembled monolayer. When a molecular target binds to the aptamer, a conformational change occurs which produces a distinct change in electron transfer from the reporter. This, in turn, produces a change in electrochemical signal easily monitored and correlated to concentration.

Electrochemical aptamer-based sensors are particularly amenable to a field setting because they respond rapidly even in complex media. Unlike many platforms, they don't require sample preparation, such as filtration, centrifugation, labeling, or dilution. And because their signaling persists even under exposure to biomolecules, they work directly in undiluted clinical samples, including whole blood. For low molecular weight targets, such as small molecule pharmaceuticals and drugs of abuse, target binding to the aptamer is rapidly reversible. Often, target response occurs in seconds to tens of seconds. Thus, in addition to rapidly detecting the presence of a molecule, the sensors can measure rising and falling concentrations of specific targets.

Already, these sensors have enabled the real-time measurement of a range of small molecule targets in the living body, including pharmaceuticals and drugs of abuse. Further, they have demonstrated utility in measuring metabolites, cytokines, and neurotransmitters. Aptamer sensors are of interest to the warfighter because, unlike other leading approaches, such as enzymatic sensing, aptamers exist for very wide range of species. And because of the flexibility of in vitro aptamer selection, additional aptamers may be rapidly produced for a range of emerging targets of interest.

Aptamer-based Sensing for Subdermal Monitoring:
Here, we explore the application of electrochemical aptamer-based sensors for sub-dermal monitoring. We demonstrate the substantial miniaturization of the sensing platform into electrodes thinner than a human hair, and then integrate them with ultra-fine microneedles. Using the high-toxicity small molecule drug, vancomycin, as a model system, we demonstrate sensor function directly in body temperature, undiluted whole blood. Building on this research milestone, we explore the application of the sensors when inserted sub-dermally into porcine skin as a proxy for the human skin. Because of the individual addressability of the sensing electrodes and the generalizability of the aptamer platform, this technology is capable of multiplexed, simultaneous detection of many different molecular targets. Thus, electrochemical aptamer-based sensing offers a promising route forward for non-invasive physiological monitoring and emergency-response triage.