RapTOR (Rapid Threat Organism Recognition) Biodetection System for Public Health and Biodefense

Challenge

Amid concern about bioterrorism threats to national security, there is, in addition to the need for rapid diagnostics for *known* biothreat agents, another requirement to detect, identify, and characterize *unknown* biological threats—that is, pathogenic microorganisms not previously encountered and possibly genetically engineered to increase threat and avoid detection. The technique of Next Generation Sequencing (NGS) enables analysis of such unknown pathogens at the genetic (DNA or RNA sequence) level, but only if a suitable sample of the pathogen's nucleic acids (DNA or RNA) is available. This is usually not the case in clinical specimens from infected patients, because the pathogen's nucleic acids exist against the far more abundant and complex background of host (human) nucleic acids. Further compounding the problem is the fact that most clinical specimens are "contaminated" with nucleic acids derived from nonpathogenic microorganisms that live in symbiotic (often mutualistic) relationships with humans, such as the hundreds of bacterial species that populate the human intestine; recently the terminology "human microbiome" has been used to describe this collection of microorganisms normally found in and on the human body. Therefore, significant amounts and wide varieties of microbial nucleic acids are present not only in specimens from GI and respiratory systems, but also even in "more-sterile" environments such as blood. Thus, when clinical samples are analyzed using a "brute-force NGS" approach (sequencing the entire collection of nucleic acids recovered from a patient sample, for example), the vast majority of DNA and RNA sequences (genetic information) are uninformative, because most belong to either human cells or to the human microbiome, with vanishingly few deriving from the nucleic acids of either known or genetically altered or unknown pathogens. This "needle in a haystack" problem boils down to wasted time and resources, making it highly unlikely that a pathogen's nucleic acids will be discovered quickly—in time to characterize the nature and biological activity of the pathogen in causing morbidity and mortality in human populations.

Research

The goal of the project is to develop and demonstrate new strategies, methods, and technologies for time- and cost-efficient use of NGS for characterization of known and unknown pathogens in clinical samples. To this end, RapTOR is developing a microfluidics-based automated molecular biology (AMB) platform that enables rapid and selective purification, amplification, and formatting of pathogen-derived nucleic acids for NGS analysis. In general, the strategy is to deplete nucleic acids of human origin, and those derived from the human microbiome (those normally resident microorganisms in and on the human body). Accomplishing this will, in effect, magnify any other nucleic acids in a sample collected from a person—that is, those of any pathogenic microorganism that might be present. Such human/microbiome depletion occurs via two techniques. *Normalization* makes use of the feature of double-stranded nucleic acids (for example the DNA in human and most bacterial chromosomes) to "find" its partner (or "complementary") DNA (or RNA) strand under suitable chemical conditions. Such finding relies on the fundamental "rules" of base-pairing, whereby all the As(adenines) on one DNA strand pair with Ts (thymines) on its complementary strand, and vice-versa (in double-stranded RNA, the pairing is actually A-U(uracil); and where all the Gs (guanines) on one DNA strand pair with Cs

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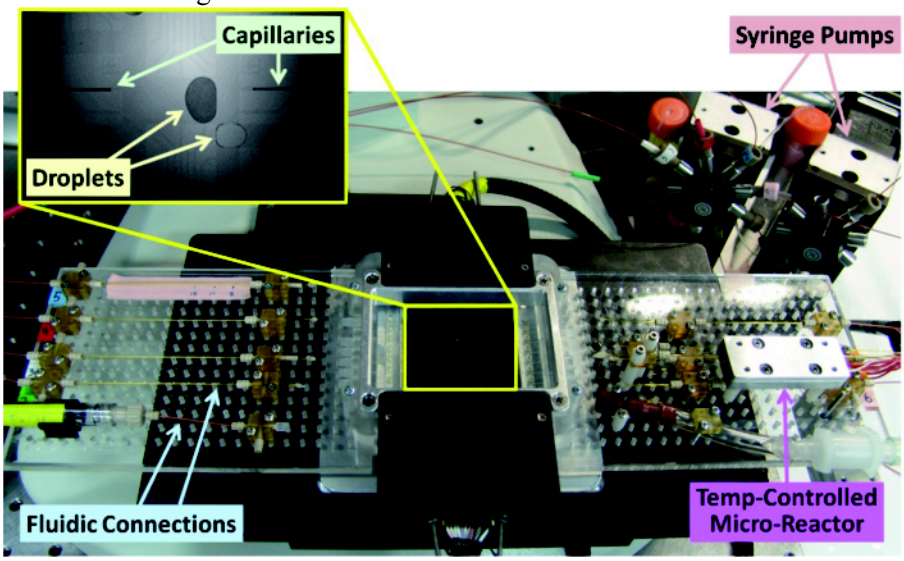
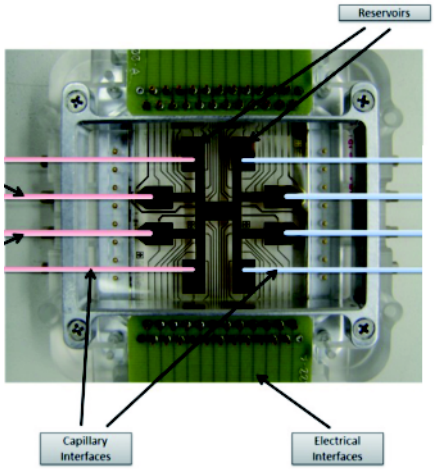
(cytosines) on its complementary strand; and vice-versa. Hence in a long stretch of double-stranded DNA that is chemically or thermally separated into its two single strands, the high likelihood is that these two "complementary" strands will "find each other" via complementary base-pairing ("re-anneal") under the right conditions. In the RapTOR project's normalization technique, double-stranded nucleic acids are dissociated at high temperature and then allowed to re-anneal at lower temperature; the most abundant double-stranded nucleic acids (typically host- and microbiome-derived) will quickly recognize and base-pair with their complementary partners, and then can be eliminated through several well-known techniques. In the capture stage of the technology, a useful technique that depends on the extremely strong affinity of the protein streptavidin for biotin (or vitamin B7)—a standard technique in molecular biology—is used to remove remaining human or microbiome nucleic acids.



Overview photo of RapTOR automated molecular biology platform.

RapTOR is capable of carrying out these processes in automated fashion, using extremely small samples, thereby reducing the requirements for amount of sample to be analyzed. This is possible due to microfluidics technology that processes microdroplets of sample at various process stages. This microfluidic processing is further integrated via a digital microfluidics (DMF)-based central hub that links together microcapillary- and microchip-based functional modules. Microdroplets are transferred from one location to another upon a Teflon-coated grid of electrodes, (*via* a process known as "electrowetting"). Microdroplets carrying various cargos (nucleic acids, reagents) can be merged and split, and the hub surface can be temperature controlled. Hence, the hub itself can execute a number of sample processing steps as well as intelligently shuttling materials to and from other sample-processing modules peripherally arrayed around the hub. Several key engineering advances include development of novel interconnects that support microfluidic communication between the hub and modules. Another important innovation was use of transparent indium-tin-oxide (ITO) electrodes for DMF, providing optical access to the microdroplets on the hub, enabling real-time monitoring of microdroplet



Closeup of fluidic & electrical interfaces

trafficking. The platform offers tremendous flexibility in the timing and ordering of processing reactions, as well as in incorporation of new functions, enabling rapid assembly and reconfiguration of a wide variety of sample processing trains.

Impact

The target for sample-to-pathogen identification turnaround is 24 hrs; providing detailed information about a known or genetically modified pathogen's genome in this timeframe would enable faster and more effective public and military responses to infectious disease outbreaks. Ongoing work to improve the AMB platform is focused on multiplexing sample processing trains and accelerating the slowest reaction step (annealing), with the goal of completing end-to-end sample processing within 4 hrs (current turnaround time is ~12 hrs). The standard bench-scale approach requires multiple workdays from a skilled technician.

To diminish biothreats to US populations requires the rapid identification not only of known pathogens, but also of genetically altered variants of these microorganisms, and identification even of sequences engineered to encode, for example, toxins that may or may not derive from any readily identifiable threat organism. This ability to rapidly isolate, sequence, and analyze pathogen-derived nucleic acids is a critical addition to the arsenal of tools to defeat biothreats. In both military and civilian situations, a few days', even a few hours' delay can easily mean the difference between the protection of small versus large percentages of exposed populations, between degrees of morbidity, and even, of course, between mortality and survival.

Beyond this most obvious and critical use, RapTOR technology has also been utilized to characterize algal pond crashes (sudden population decrements in large-scale cultures of algae for biodiesel production), as well as for human DNA fingerprinting (for purposes of unambiguous identity) in military field situations.

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