Title
DNAzyme-coupled droplet microfluidics for detecting single bacteria in blood

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Nosocomial and community-acquired infection of deadly bloodborne pathogens is a major threat to public health. Moreover, emergence of multi-drug resistant pathogens is another immense challenge to maintain public health. Accurate detection of such pathogens at the early stage can help physicians to select proper antibiotics to save lives and lighten the economical burden to our society. However, unfortunately, blood culture method, the gold standard for the detection of bacteremia, takes several days to obtain results. Molecular diagnosis methods, such as polymerase chain reaction (PCR) and immunoassays are often not sensitive enough to detect bacteria that occur at low concentrations in blood (1-100 colony-forming unit (CFU)/mL). Moreover, all these techniques are sophisticated and expensive, and therefore not well-suited for routine testing. Therefore, simple methods are urgently needed for rapid and sensitive identification of bacteria in blood, which has the potential to significantly reduce the mortality rate and the cost of medical care associated with blood stream infections.

In this study, we have developed a droplet microfluidic system that integrates bacteria-specific DNAzyme sensors to detect bacteria in patient blood at single-cell sensitivity within a few hours. Our central hypothesis is that the confinement of bacteria in droplets significantly increases the concentration of released target molecules that can activate the DNAzyme sensors to produce detectable fluorescent signal in a rapid, real-time fashion. Specifically, infected patient blood is mixed with DNAzyme sensor solution including bacteria lysis buffer within the microfluidic channel which is encapsulated into millions of individual picoliter droplets. Because bacteria exist at low numbers in blood, we anticipate that each droplet contains one or no bacteria. DNAzyme sensors fluoresce instantaneously in the droplets that contain bacterium. The droplets are monitored by APD (avalanche photodiode) embedded confocal microscopy in a high throughput manner.

Our novel approach of integrating real-time DNAzyme sensors with droplet microfluidics bypasses many challenges faced by current techniques (e.g., blood culture) such as culture, isolation of genomic DNA which is required for PCR. This rapid detection and early intervention will therefore be significantly helpful of treating blood stream infections and reduce mortality.