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RAPID, SINGLE BACTERIAL DETECTION FROM BLOOD USING MICROENCAPSULATED SENSORS

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ABSTRACT

We report herein a platform technology called 'microencapsulated sensors' that is able to selectively detect bacteria in unprocessed whole blood at single-cell sensitivity in a one-step, homogenous, and culture- and amplification-free reaction. Our system integrates real-time DNAzyme sensor technology and droplet microfluidics.

KEYWORDS: Droplet Microfluidics, Microfluidics, Single Bacteria Detection, Microencapsulation

INTRODUCTION

Blood stream infections (BSIs) are a major cause of morbidity and mortality. Sepsis resulting from a BSI annually affects over 18 million people worldwide and 700,000 in the U.S., with a mortality rate of 30-40% [1, 2]. Sepsis and other aggressive bacterial infections associated with BSIs are often times managed within intensive care units with associated high costs, which impose significant healthcare, economic and social burdens. For instance, each septic patient in the US incurs costs of approximately $ 25,000 during hospitalization, corresponding to $17 billion annually [1, 2]. The extremely high mortality of blood infections is due, in part, to the inability to rapidly detect, identify and thus treat bacteria with appropriate antibiotics in the early stages of infection. The initial treatment with empirical broad-spectrum antibiotics not only is inadequate but also encourages antibiotic resistance [4, 5]. It is widely recognized that effective detection and monitoring of patients to diagnose a BSI at an early-stage have a profound effect on survival rates [1-3]. However, the present gold standard to detect a BSI is blood culture, which unfortunately can take days to get a definitive result. Recent amplification-based molecular diagnosis methods including polymerase chain reaction (PCR) can reduce the assay time to hours but are often not sensitive enough to detect bacteria that occur at low concentrations in blood (<1 to 100 colony-forming unit (CFU)/mL) as is commonly found in adult BSIs and therefore often still require a culture-enrichment step. Moreover, these conventional methods typically suffer from poor specificity and high background because a target bacteria is surrounded by billions of non-target species (e.g., red blood cells) in blood sample. More recent nano- and micro-systems including droplet microfluidics (e.g., digital PCR) can improve detection sensitivity and selectivity but typically are limited to small sample volume (μLs), which can not handle the required clinical sample volume (mLs blood) and throughput. Inevitably, the existing methods typically require expensive equipment and lengthy, complex sample processing (e.g., cell lysis, nucleic acid extraction, centrifugation, magnetic separation, washing and signal amplification) for target purification and enrichment, which not only results in significant loss of rare target organisms, and therefore contributes to a high false-negative rate, but also limit their widespread use especially in a point-of-care setting [6]. We present herein a platform technology called microencapsulated sensors that is able to selectively detect bacteria in mLs of unprocessed whole blood at single-cell sensitivity in a one-

step, homogenous, and culture- and amplification-free reaction within 1-3 hours. Our system integrates real-time DNAzyme sensor technology and droplet microfluidics.

EXPERIMENTAL

DNAzyme sensors used here are short catalytic oligonucleotides that are identified by in vitro evolution to selectively react with target bacteria, leading to a rapid, real-time fluorescence signal (Figure 1a). Specifically, blood samples are mixed with the DNAzyme sensor solution including bacteria lysis buffer within a microfluidic channel, which is then immediately encapsulated into 100s of millions of individual picoliter droplets. The confinement of bacteria in droplets that serve as "microreactors" significantly increases 1) the concentration of released target molecules such that single bacterium can be detected by the DNAzyme sensors in a rapid, real-time fashion, and 2) target/background ratio to minimize interference from nonspecific binding and noise. Droplets will be analyzed using a high throughput & highly sensitivity confocal microscopy containing avalanche photodiode (APD) (Figure 2a).

RESULTS AND DISCUSSION

In this study, we have developed a system that is able to detect bacteria in patient blood with single-cell sensitivity within a few hours. Our system integrates bacterium-detecting DNAzyme sensors (Figure 1a), which are obtained by in vitro selection, with droplet microfluidics (Figure 2a). Our central hypothesis was that the confinement of bacteria in droplets significantly increases the concentration of released target molecules that can be detected by the DNAzyme sensors in a rapid, real-time fashion. Specifically, infected patient blood was mixed with DNAzyme sensor solution, including bacteria lysis buffer, within the microfluidic channel, which was encapsulated in millions of individual picoliter droplets (Figure 3). Because bacteria exist at low numbers in blood, we anticipated each droplet will contain one or no bacteria. DNAzyme sensors fluoresced instantaneously in the droplets that contain bacterium. The droplets were monitored by Avalanche Photodiode (APD).

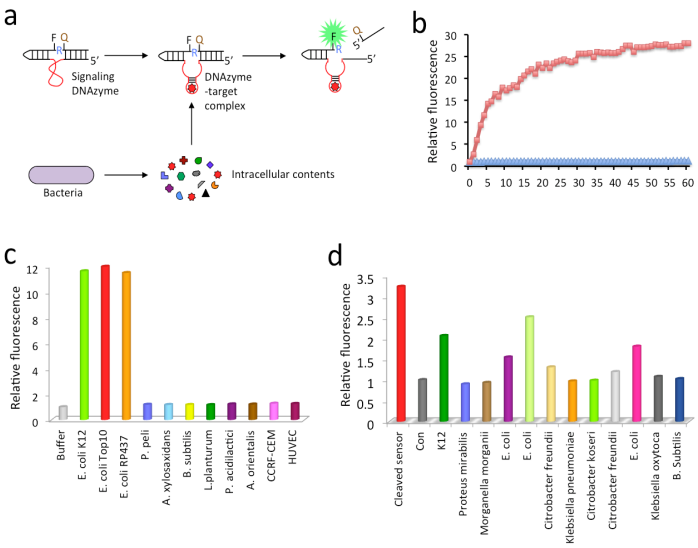

Figure 1: E.coli detecting DNAzyme sensor. (a) Mechanism of how the DNAzyme sensor generates fluorescence signal upon interaction with the target molecules secreted from bacteria (F is Fluorescein-dT; Q is Quencher-dT). (b) Activity of DNAzyme sensor with target E.coli. (c) DNAzyme sensor selectively detects E. coli. (d) Clinical isolated E.coli is specifically identified with DNAzyme sensor among other clinical isolates.

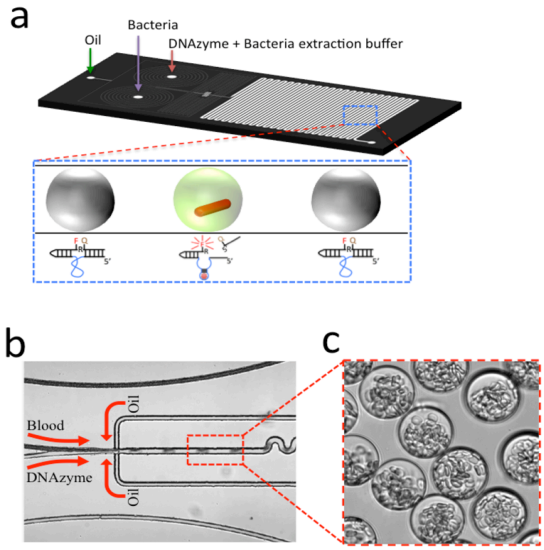

Figure 2: Blood samples and DNAzyme sensors are encapsulated by droplet-based microfluidic device. (a) Layout of droplet-based microfluidic device and cleavage reaction of DNAzyme in droplets. (b) Microencapsulation of blood with DNAzyme sensor using droplet-based microfluidic device. (c) Uniform microdroplets (30 μm) containing blood components and sensor solution are being formed (20% final blood content).

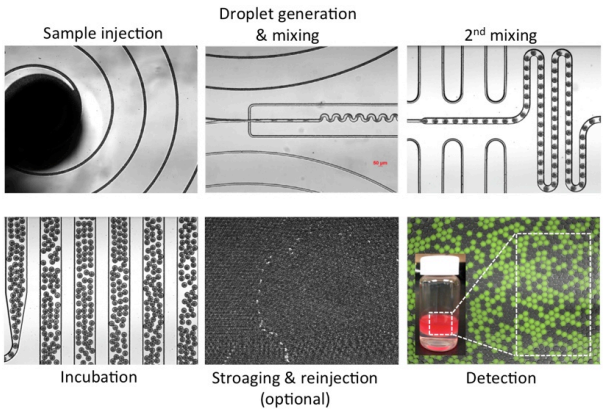

Figure 3: Work flow of bacteria detection in blood. Blood samples and DNAzyme sensors are introduced into the device through each inlets and microencapsulated by flow-focusing structure. Droplets are then mixed and collected into the vial to identify bacteria

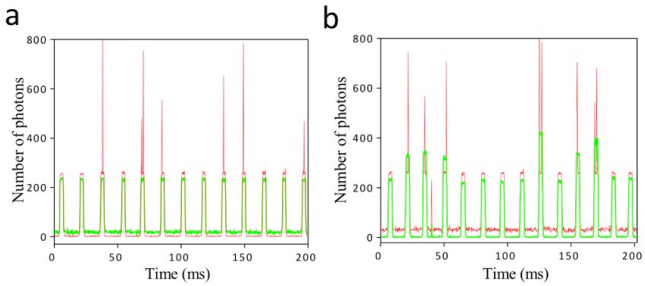

Figure 4: SYTO 17 (red color) stained control Bacillus (a) or target E. coli (b) were spiked in blood which was encapsulated in a single cell manner in droplets with DNAzyme sensor (green color) (final blood content is 10% in this data). After reaction, droplets are counted on-chip using our confocal detection system. Red spikes represent droplets that contain SYTO 17 stained cells which are observed on both control (a) and target (b) cells. However, only the target E. coli (b) produced a green color DNAzyme signal that is above the background (i.e., droplets that do not contain cells).

CONCLUSION

Our novel approach of integrating real-time DNAzyme sensors with droplet microfluidics bypasses many challenges faced by current techniques (e.g., blood culture). This rapid detection and early intervention will therefore significantly improve the chances of treating blood stream infections and reduce mortality.

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