Biosensors and Bioelectronics 97 (2017) 260–266

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Accelerating bacterial growth detection and antimicrobial susceptibility assessment in integrated picoliter droplet platform

Aniruddha M. Kaushik[a,1], Kuangwen Hsieh[a,1], Liben Chen[a], Dong Jin Shin[a], Joseph C. Liao[b], Tza-Huei Wang[a,c,*]

[a] Department of Mechanical Engineering, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD, USA
[b] Department of Urology, Stanford University School of Medicine, 300 Pasteur Dr. S-287, Stanford, CA, USA
[c] Department of Biomedical Engineering, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD, USA

ARTICLE INFO

Keywords:
Droplet
Microfluidics
Antimicrobial Susceptibility Test (AST)
Picoliter

ABSTRACT

There remains an urgent need for rapid diagnostic methods that can evaluate antibiotic resistance for pathogenic bacteria in order to deliver targeted antibiotic treatments. Toward this end, we present a rapid and integrated single-cell biosensing platform, termed *dropFAST*, for bacterial growth detection and antimicrobial susceptibility assessment. DropFAST utilizes a rapid resazurin-based fluorescent growth assay coupled with stochastic confinement of bacteria in 20 pL droplets to detect signal from growing bacteria after 1 h incubation, equivalent to 2–3 bacterial replications. Full integration of droplet generation, incubation, and detection into a single, uninterrupted stream also renders this platform uniquely suitable for in-line bacterial phenotypic growth assessment. To illustrate the concept of rapid digital antimicrobial susceptibility assessment, we employ the dropFAST platform to evaluate the antibacterial effect of gentamicin on *E. coli* growth.

1. Introduction

The emergence of multi-drug resistant bacteria due to indiscriminate use of broad-spectrum antibiotics has grown into a global healthcare crisis (Boucher et al., 2009; Carlet et al., 2011; Finley et al., 2013; Kunin et al., 1973; Roca et al., 2015). To combat this threat, there is an urgent need for diagnostic methods that can rapidly evaluate antibiotic resistance of these dangerous bacteria to ensure timely and targeted antibiotic treatments (Exec. Order No. 13676, 3 C.F.R., 2014; Laxminarayan et al., 2013; Spellberg et al., 2011). To this end, various genetic techniques have been proposed based on amplification and detection of known resistance-conferring genes in bacteria (Tenover, 2010). However, due to constantly evolving genetic mechanisms of resistance, such approaches are less reliable than direct phenotypic detection of bacterial growth (Mach et al., 2011). Phenotypic methods for evaluating antibiotic resistance, in which bacteria are directly grown in the presence of various antibiotics to determine their sensitivity or resistance, are limited by a lengthy assay procedure. Indeed, current standard phenotypic antimicrobial susceptibility tests (AST) in clinical laboratories (e.g., broth dilution and disk diffusion) require a 16–20 h incubation period (Davenport et al., 2017; Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement, 2014). Although many strains of bacteria grow rapidly and some (e.g., *E. coli*) can even replicate in as little as every 20 min (Fossum et al., 2007; Parija, 2016; Powell, 1956), conventional AST still requires a lengthy incubation period. This is in part attributed to the large volumes in which bacteria are cultured and measured (100 µL to 20 mL), which necessitate growing a high number of bacteria to ensure reliable interpretation of bacterial growth. This observation suggests that a reduction of analysis volume can reduce the incubation time necessary for assessing antibiotic resistance. Moreover, this incubation time may be further reduced to the timescale of individual bacterial replication if such an event can be reliably observed at the single-cell level. In short, a technology capable of handling small sample volumes and detecting the replication of individual bacteria can drastically accelerate phenotypic assessment of antimicrobial susceptibility.

Microfluidics is well-suited for handling reaction volumes at microliters and below. Measuring reactions confined in small volumes benefits from high local signal-to-background ratio, which can accelerate single-cell detection and measurements. Indeed, researchers have reported various microchambers (Balaban et al., 2004; Choi et al., 2013; Mohan et al., 2013; Sun et al., 2011) and microchannels (Chen et al., 2010; Li et al., 2014; Lu et al., 2013; Mai et al., 2014; Wakamoto

* Corresponding author at: Department of Mechanical Engineering, Johns Hopkins University, Shaffer Hall 200A, 3400 N. Charles St., Baltimore, MD, USA.
E-mail address: thwang@jhu.edu (T.-H. Wang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bios.2017.06.006
Received 29 January 2017; Received in revised form 27 April 2017; Accepted 5 June 2017
0956-5663/ © 2017 Elsevier B.V. All rights reserved.

et al., 2013) that can couple with microscopy to detect the growth and replication of single bacterial cells. Unfortunately, with throughputs limited to the order of tens of channels and chambers per device, these devices lack the statistical capacity to analyse many cells and account for the inherent heterogeneity of the bacterial population (Delvigne et al., 2014; Nichols et al., 2011). Furthermore, these devices frequently rely on additional materials (e.g., cellulose membranes (Balaban et al., 2004; Wakamoto et al., 2013) and agarose gels (Choi et al., 2013; Eun et al., 2011; Li et al., 2014)) or external forces (e.g., electric force (Lu et al., 2013)) to trap bacteria, which increase the complexity of device fabrication and operation. In part to address such challenges, droplet microfluidics recently emerged as an approach for single-cell isolation, detection, and analysis (Kaminski et al., 2016; Yan et al., 2016). Similar to microchambers and microchannels, microfluidic droplets enable volume reduction, high local signal-to-background ratio, and accelerated time to detection (Boedicker et al., 2008; Ng et al., 2016; Rane et al., 2012a, 2012b). Critically, droplet-based platforms employ passive "flow-focusing" structures to generate droplets that can stochastically encapsulate single bacteria at around 100–1000 Hz and detection modules that can exclude empty droplets from analysis. Droplet-based platforms therefore offer a compelling alternative for accelerating single-cell detection and measurements.

Despite these promising attributes, droplet microfluidics has not been able to reduce the analysis time of antimicrobial susceptibility assessment to the timescale of bacterial replication. In an early example, Boedicker et al. (2008) tested antimicrobial susceptibility of *S. aureus* after incubating the bacteria for 7 h in the presence of antibiotics via a single-step fluorescence assay. The relatively large droplet volume of 4 nL in this work necessitated 7 h incubation for reliable growth detection. In a later work, Liu et al. (2016) used smaller droplets of 330 pL for detecting a mutant resistant to fusidic acid, but required 8 h incubation due to a less-sensitive non-fluorescent detection approach based on light scattering. Notably, these techniques were demonstrated on experimental workflows requiring a separate device for droplet generation, an incubator for off-chip droplet incubation, and an additional device for droplet detection (Boedicker et al., 2008; Liu et al., 2009, 2016). To accelerate the analysis time for assessing antimicrobial susceptibility to the timescale of bacterial replication, an integrated workflow that ensures precise temporal control from single-bacteria confinement to detection must also be implemented.

Herein, we present a rapid and integrated method for single-bacteria growth analysis and antimicrobial susceptibility assessment, *dropFAST* (droplet-based Fluorescent Antimicrobial Susceptibility Test). In dropFAST, we encapsulate and incubate single bacterial cells in picoliter-sized droplets that are more than ten-fold smaller than those reported in earlier works, and we use a fluorescence assay for detection of bacterial growth. This combination allows us to substantially reduce the required time for detecting signal from growing bacteria to approach the fundamental limit of growth determination – the bacterial replication time. Importantly, the shortened incubation time minimizes the device footprint, which facilitates the integration of single bacterial cell encapsulation, bacteria culturing, and fluorescence-based bacterial growth detection into a streamlined workflow within a monolithic device. As an additional benefit, dropFAST routinely analyzes ~ 10^4–10^5 individual bacterial droplets in each experiment; such throughput confers the statistical capacity to account for potential inaccuracies due to bacterial population heterogeneity. As a demonstration, we assessed the antibacterial effect of the antibiotic gentamicin on the growth of *E. coli* with single-cell resolution after only 1 h of on-chip incubation.

2. Materials and methods

2.1. Design and fabrication of microfluidic device

The microfluidic device design consists of a 20 µm flow-focusing nozzle for droplet generation, followed by a 500-µm-wide serpentine incubation channel that spans roughly 1.75 m and then a 10 µm droplet detection window. A casting mold was fabricated by spinning a 20 µm layer of SU8-3050 photoresist (MicroChem, Westborough, MA) onto a 4 in. silicon wafer and patterning using standard photolithography. The microfluidic devices were made of polydimethylsiloxane (PDMS) by pouring 30 g of 10:1 ratio of Sylgard 184 (Dow Corning, Auburn, MI) base to curing agent onto the SU8 mold. After curing the PDMS replica, it was permanently bonded to cover glass (130 µm thickness, Ted Pella, Redding, CA) through oxygen plasma treatment in order to seal the channels. Prior to operation, the microfluidic chips were treated with Aquapel (Pittsburgh Glass Works, LLC, Pittsburgh, PA) and baked at 80 °C for ≥ 20 min to render microfluidic channel surfaces hydrophobic.

2.2. Operation of microfluidic device

Frozen stocks of *E. coli* (ATCC 25922, ATCC BAA 2471) were thawed, washed twice, and diluted to 10^7 CFU/mL in Muller-Hinton II cation adjusted broth (Sigma-Aldrich, St. Louis, MO). Separately, 400 µM resazurin (Sigma-Aldrich) was mixed with either 0 µg/mL or 8 µg/mL gentamicin (Sigma-Aldrich) in Mueller-Hinton broth. Both bacterial sample and resazurin/antibiotic solution were then drawn into separate 1-m-long sections of Tygon tubing (Cole-Parmer, Vernon Hills, IL) with an inner diameter of around 500 µm. Both Tygon tubing sections were individually connected to Hamilton 1000 glass syringes (Sigma-Aldrich) containing FC-40 oil (Sigma-Aldrich), which served as the displacement fluid for injecting both aqueous samples from Tygon tubings into the device using a syringe pump at 15 µL/h (Harvard Apparatus, Holliston, MA). An oil phase consisting of FC-40 oil and 5% poly(ethylene glycol) di-(krytox-FSH amide) surfactant by weight was pumped into the device through the oil inlet of the device at 60 µL/h by a separate syringe pump. To confirm stable droplet generation, the device was imaged using a 4× objective lens and a CCD camera during droplet generation and after droplet incubation. Droplet incubation was conducted on chip at 37 °C using a controllable peltier heater on which the microfluidic device rested.

2.3. Continuous-flow droplet detection

Continuous-flow droplet detection was conducted using a custom optical stage consisting of a 552 nm laser excitation source (OBIS, Coherent, Inc., Santa Clara, CA) and a silicon avalanche photodiode detector (APD) (SPCM-AQRH13, ThorLabs, Newton, NJ). The laser was operated at 1 mW power and was focused into the detection zone of the device using a 40× objective (Thorlabs RMS40X-PF, NA 0.75, focal depth ~ 0.6 µm). As droplets flowed through the detection zone, fluorescence data was continuously acquired by the APD with 0.1 ms sampling time. A custom LabVIEW program was used to control fluorescence data acquisition.

Download English Version:

https://daneshyari.com/en/article/5031009

Download Persian Version:

https://daneshyari.com/article/5031009

Daneshyari.com