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Short communication

High-throughput microarray for antimicrobial susceptibility testing

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ABSTRACT

We describe the development of a novel, high-throughput, nano-scale microarray platform for antimicrobial susceptibility testing (AST). The platform allows to process 480 samples at 50 nL volume on a single chip, analyze by fluorescence read-out with an easy dunk-and-rinse step, and the ability to process multiple samples and chips simultaneously. We demonstrate the applicability of this chip for culturing community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA), and perform AST against clinical isolates of CA-MRSA. The chip platform holds promise for an impact in microbial biotechnology as an attractive high-throughput, lower sample volume and quicker alternative to conventional AST such as the traditional broth microdilution or the newer automated systems.

1. Introduction

Antimicrobial resistance remains a major threat to human health throughout the world, largely due to the indiscriminate use of antibiotics. The liberal use of antibiotics is driven by the need to quickly administer active antimicrobial therapy in the face of diagnostic uncertainty. AST is commonly performed using principles based on broth microdilution or disk diffusion assays, and takes 2-3 days, which includes the time for culturing the clinical isolates followed by antibiotic susceptibility testing by exposing the cultures to a panel of antibiotics over a range of concentrations [1]. Typically, clinical samples isolated from patients are cultured to increase the inoculum and isolate the pathogen; and then the isolates are exposed to a range of antibiotic concentrations either in 96-well plate suspension cultures or drug-filled cassettes (broth microdilution) or to a single concentration of the drug-impregnated disc placed on an agar plate containing the organism (disk diffusion). Susceptibility is then assessed by visual inspection either manually or by an automated instrument. Thus, it is conceivable that obviating relatively large culture volume or cell density may increase the throughput, and decrease the time required to obtain AST results. It is now well established that faster availability of AST data can enable the clinician to initiate or switch out of broad-spectrum treatment regimen to appropriate antimicrobial therapy sooner, and significantly reduce health care costs and morbidity [2]. While there has been some progress in the recent years for rapid AST [3–5], the currently available tests are either limited to a few select organisms such as PCR-based methods [6,7], may not predict drug response reliably [8–10], limited in throughput or are cost-prohibitive such as MALDI-TOF MS or NMR [11] to most labs even in developed countries. To address some of the aforementioned issues, we have designed a novel microarray platform for high-throughput antimicrobial susceptibility testing (AST-Chip) and tested against select community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) isolates.

2. Materials and methods

2.1. Culture conditions

Frozen stocks of *Staphylococcus aureus* strain UAMS 1 and clinical isolates were subcultured onto tryptic-soy-agar plates (TSA) (BD Difco, MD, USA), and propagated the in 10 ml of tryptic soy-broth (TSB) (BD Difco, MD, USA) in an orbital shaker at 37 °C. In order to capture cells in log-phase, 100 µl from the overnight liquid culture were subcultured into 10 ml of TSB for 3 h.

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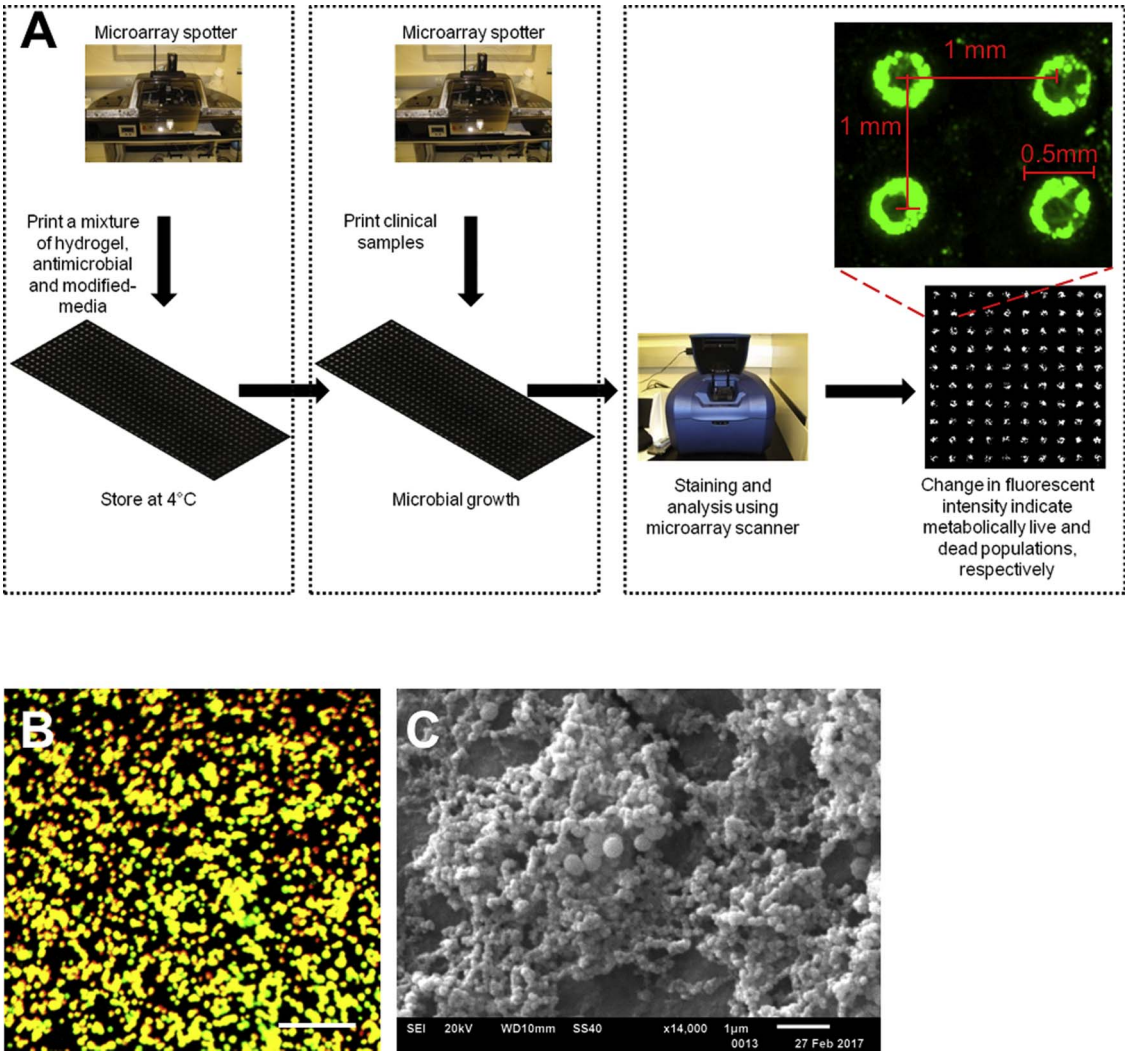

Fig. 1. High-throughput AST chip. (A) A schematic of high-throughput antimicrobial susceptibility testing using AST-Chip. Antimicrobial susceptibility testing using the chip can be conducted in 12 h; Microarray micrograph of *S. aureus* cultures formed on hydrogel spots of AST-Chip after 12–18 h. The spots are interspaced at 1 mm with a spot diameter of 0.5 mm; The nano-biofilms analyzed using FUN-1 is demonstrated in green color; (B) BacLight live/dead assay shows metabolically active cells in yellow-orange color, present as colonies. The scale bar is 100 μm; (C) SEM micrograph of *S. aureus* nano-biofilm colonies. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Preparation of AST-Chip

The biofilm chip was prepared by modifying glass slides, and the growth conditions were optimized to obtain maximum biofilm yield of *S. aureus* using factorial design as described in [12,13]. The optimized media is a concoction of 2X Yeast Peptone Dextrose (YPD), 3X Brain Heart Infusion (BHI) along with 10% human serum. Antimicrobial stock solutions of doxycycline, vancomycin hydrochloride (Sigma, MO, USA), and clindamycin hydrochloride (RPI Corp., IL, USA), were diluted in phosphate buffered saline to a maximum concentration of 100 μg/ml. Any subsequent dilutions needed for the antimicrobial susceptibility assays were made in PBS, mixed in a suspension of 0.5% alginate, and optimized media, and 50 nL of this mixture was spotted using a non-contact microarray spotter (Omnigrid Micro, Digilab Inc., Holliston, MA) on modified glass slides. An array of 40 rows and 12 columns was printed at room temperature with relative humidity of 100%. The AST-chip was stored at 4 °C for up to one week before use.

2.3. S. aureus nano-biofilms and viability assay

S. aureus cells were adjusted to a density of 1×10^7 cells/ml in modified growth medium, and printed on top of the drug spots. After printing, the slides were then placed in a humidified hybridization cassette (Arrayit, Sunnyvale, CA, USA) to prevent evaporation of spots and incubated at 37 °C. All microarrayer functions such as sample loading, priming, printing, and spatial distribution of the array were controlled by AxSys programming (Digilab, MA, USA). The viability of nano-biofilms on the microarray was determined by staining with FUN-1 fluorescent dye as described in [13,14]. The fluorescence from the chips were measured using a microarray scanner (GenePix Personal 4100A; Axon Instruments, Union City, CA), and the images were analyzed with GenePix Pro V7 (Axon instruments, Union City, CA) to determine the antimicrobial susceptibility by normalizing the intensity between 0% and 100% for dead and live cells, respectively. The susceptibility values were obtained as average of 20–30 spots for each treatment from a single chip, and this experiment was performed in duplicate on two different days. The IC50 values were calculated using GraphPad Prism [15].

2.4. Microscopy

Fluorescence microscopy of *S. aureus* biofilms was performed after staining the slides with BacLight (Life Technologies) following manufacturer's protocol, and visualized at 40X (DMI6000, Leica). Scanning