Lab on a Chip

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c5lc00375j

Rapid electrochemical phenotypic profiling of antibiotic-resistant bacteria†

Justin D. Besant,[a] Edward H. Sargent*[b] and Shana O. Kelley*[acd]

Received 28th March 2015,
Accepted 13th May 2015

DOI: 10.1039/c5lc00375j

www.rsc.org/loc

Rapid phenotyping of bacteria to identify drug-resistant strains is an important capability for the treatment and management of infectious disease. At present, the rapid determination of antibiotic susceptibility is hindered by the requirement that, in existing devices, bacteria must be pre-cultured for 2–3 days to reach detectable levels. Here we report a novel electrochemical approach that achieves rapid readout of the antibiotic susceptibility profile of a bacterial infection within one hour. The electrochemical reduction of a redox-active molecule is monitored that reports on levels of metabolically-active bacteria. Bacteria are captured in miniaturized wells, incubated with antimicrobials and monitored for resistance. This electrochemical phenotyping approach is effective with clinically-relevant levels of bacteria, and provides results comparable to culture-based analysis. Results, however, are delivered on a much faster timescale, with resistance profiles available after a one hour incubation period.

Introduction

The overuse of antibiotics and the prescription of antibiotics to which a pathogen is not susceptible contribute to rising antibiotic resistance rates – a growing threat to public health worldwide.[1] Urinary tract infections are among the most prevalent bacterial infections.[2] Gold-standard antibiotic susceptibility tests for urinary tract infections rely on culture and require 1–3 days in order to allow the bacteria to multiply to detectable levels.[3] After pre-culture of the bacteria, an additional 18 hours are typically required to perform standard susceptibility tests. Reducing the time needed to determine the susceptibility profile of urinary tract infections could improve clinical outcomes, especially in the case of the most severe infections that lead to urosepsis.[4] Rapid testing could also contribute to decreased unnecessary antibiotic use,[5] and could increase the efficiency of centralized diagnostic laboratories.

Tests for antibiotic resistance that rely on enzymatic amplification of antibiotic-resistance genes reduce turnaround times compared to culture.[6–9] Unfortunately, these assays often require a pre-incubation step to allow the bacteria to multiply, and, further, often require several hours to amplify the genes of interest. Gene-based assays are also limited by the requirement of knowing *a priori* which genes confer resistance. Dozens of constantly-evolving genes may be implicated in resistance to a given antibiotic, and it is impractical to test for all possible mutations simultaneously.[10]

Assays that monitor bacterial viability in response to antibiotics overcome the limitations of genetic tests. These tests report directly on the question of greatest clinical importance: whether a given antibiotic decreases bacterial survival. New assays for antibiotic resistance include the detection of bacterial motion using AFM cantilevers,[11] electrochemical measurements of bacterial growth,[12–16] optical detection of bacterial growth,[17,18] and optical detection of redox reporters of bacterial metabolism.[19–22] In assays that detect metabolically-active pathogens, the bacteria are incubated with the antibiotic and a redox reporter of metabolism such as resazurin or methylene blue. Metabolically-active bacteria create a reducing environment and either directly or indirectly reduce the compound, and the change in redox state is read out as a change in color or fluorescence. Resistant bacteria continue to multiply and metabolize the compound, while susceptible bacteria do not.

Successful detection using this type of approach hinges on the requirement that a sufficient quantity of the reduced form of the reporter compound accumulates above the detection threshold, a delay that takes at least 12 hours in milliliter-scale culture.[19] Strategies have been proposed that seek to confine bacteria in microliter and nanoliter volumes with the goal of reducing the time of detection by increasing

[a] Institute for Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, M5S 3G9, Canada. E-mail: shana.kelley@utoronto.ca
[b] Department of Electrical and Computer Engineering, Faculty of Engineering, University of Toronto, Toronto, ON, M5S 3G4, Canada. E-mail: ted.sargent@utoronto.ca
[c] Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, M5S 3M2, Canada
[d] Department of Biochemistry, Faculty of Medicine, University of Toronto, Toronto, ON, M5S 1A8, Canada

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5lc00375j

the local concentration of the bacteria.[20,21,23–25] In the most sensitive of these optical techniques, the sample is divided into millions of nanoliter droplets and the signal is readout sequentially from each droplet with a high-powered fluorescence microscope.[20,21,25] Despite the increase in local effective concentration provided by this approach, several hours are still required for analysis. Moreover, many of these devices only detect the presence or absence of a pathogen and not its antibiotic susceptibility profile.[25–27]

Despite several recent advances in ultrasensitive electrochemical detection of bacteria,[28–30] few devices have been reported for direct electrochemical detection of antibiotic resistance. Electrochemical readout requires only simple electronics allowing direct electronic detection of antibiotic susceptibility from confined nanoliter droplets without complex optical instrumentation for readout. Here, we describe a new strategy for rapid electrochemical phenotyping that effectively identifies the antibiotic susceptibility profile of bacteria. We describe the development of an assay that uses electrochemical readout to detect metabolically active bacteria. The electrochemical reduction of resazurin is monitored to establish the presence of live bacteria, and analyzed in the presence of antibiotics to determine resistance profiles. We utilize this assay in conjunction with a novel device to concentrate and incubate bacteria in an array of miniaturized culture chambers (Fig. 1). Captured bacteria are incubated with antibiotics and a redox reporter of bacterial viability. Small changes in the redox state of the dye can be rapidly detected using in-well electrodes (Fig. 1B). Using this assay, we detect a clinically relevant concentration of bacteria with a 30 minute incubation. Finally, we show that the antibiotic susceptibility profile of a clinically-relevant concentration of bacteria in urine can be determined after an one-hour incubation without any other pre-incubation steps. This is the first approach that provides antibiotic resistance phenotyping on such a short time scale.

Experimental

Culture device preparation

Gold electrodes were patterned using standard photolithography on glass substrates. A passivating SU-8 2002 (Microchem, Newton, MA) layer was patterned to insulate the electrodes. SU-8 3050 was patterned to create the 50 μm tall channel and wells. A final SU-8 2002 layer was patterned to form the in-well barrier used to trap microbeads. The working electrodes were electroplated by applying −300 mV for 30 s with respect to an Ag/AgCl reference electrode in a solution of 50 mM $HAuCl_4$ and 0.5 M HCl. Holes were punched in a polydimethylsiloxane (PDMS) lid for the inlet and outlet. The PDMS lid

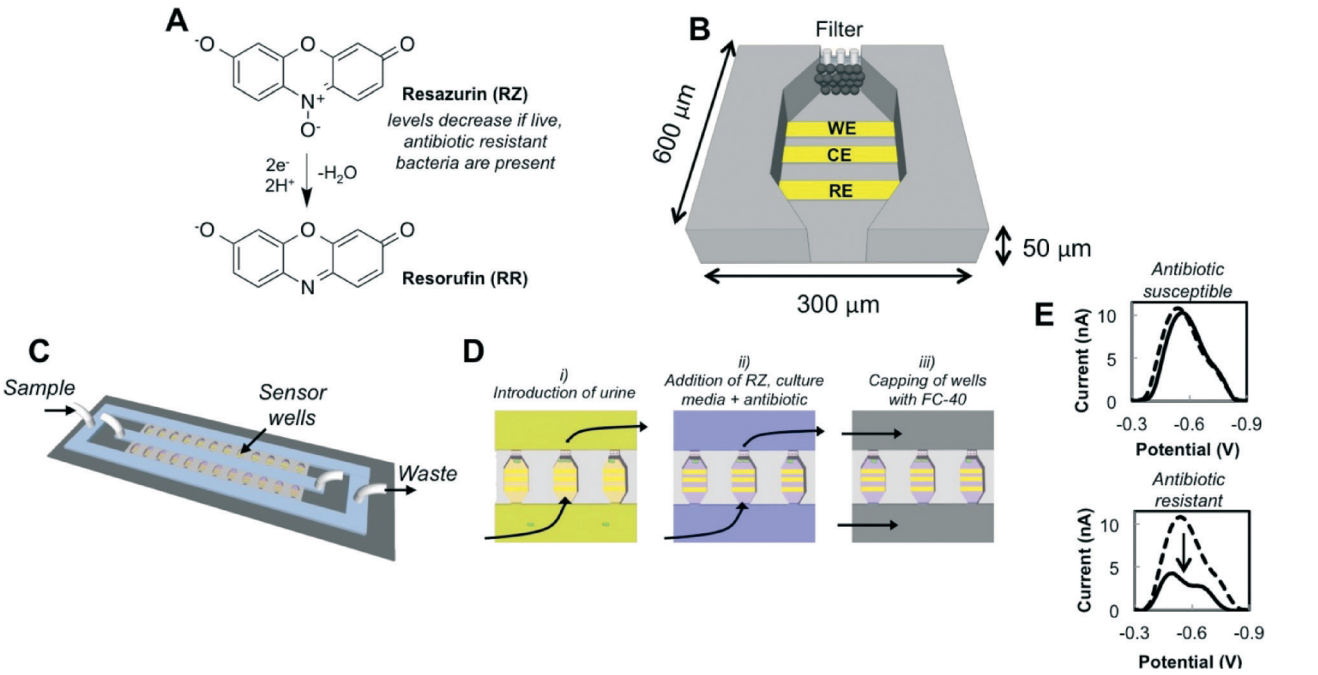

Fig. 1 Overview of the electrochemical phenotypic testing approach. (A) Resazurin (RZ) is reduced to resorufin (RR) by metabolically active bacteria. (B) Schematic of a single well containing a working (WE), counter (CE) and reference electrode (RE). Each well also contains an integrated filter for bacterial capture. (C) Schematic of the antibiotic susceptibility testing device. The bacteria are cultured in miniature culture chambers, each of which contains a filter for bacterial capture and electrodes for readout of bacterial metabolism. (D) i. A urine sample containing bacteria is introduced into the device and bacteria are captured within wells. ii. Resazurin, the culture media, and an antibiotic are introduced. iii. Plugs are formed within wells by introducing an immiscible oil. (E) Representative differential pulse voltammograms (DPVs) illustrating the principle of electrochemical phenotyping. Bacteria are cultured within the wells with resazurin and an antibiotic. Susceptible bacteria do not reduce the compound, while resistant bacteria reduce resazurin to resorufin. The two states of the molecule can be distinguished by in-well electrodes. See Fig. S1–S3 in ESI† for further electrochemical characterization of resazurin.

was bonded to the top of the device after an oxygen plasma treatment for 30 s.

To remove bubbles, the device was initially filled with EtOH and flushed with phosphate buffered saline (PBS). 100 μL of microbeads (Sigma Aldrich, St. Louis, MO) with a 5 μm diameter diluted 1 : 100 in PBS were introduced at 10 μL min^{-1} to form the in-well filters.

Bacterial culture

GFP *E. coli* (Invitrogen, Carlsbad, CA), *E. coli* (ATCC 700928) and *K. pneumoniae* (ATCC 700603) were cultured in an incubating shaker at 33 °C in LB and Nutrient Broth respectively. Concentrations were determined using optical density measurements at 600 nm using a UV-vis spectrometer (Agilent, Santa Clara, CA). These measurements were correlated with the number of colonies on agar plates incubated overnight.

Determination of bacterial capture efficiency

A 100 μL volume of serial dilutions of *E. coli* were introduced into the capture device at 10 μL min^{-1}. After capture, the device was washed with 100 μL of PBS buffer. Finally the bacteria were eluted in sterile PBS buffer. The eluted volume was plated on LB agar plates overnight at 37 °C and the colonies were counted.

Electrochemical detection of bacteria

Serial dilutions of *E. coli* were spiked in buffer and introduced into the chip at 20 μL min^{-1} followed by 200 μL of 1 mM resazurin in LB broth. Air was flushed through the device to form the wells followed by FC-40, a fluorinated oil. The device was incubated in a water bath at 37 °C.

Antibiotic susceptibility microdilution assay

Cultured *E. coli* and *K. pneumoniae* were diluted to 100 cfu μL^{-1} and incubated at 37 °C in a 96 well plate in Nutrient Broth with serial dilutions of ciprofloxacin and ampicillin. After 24 hours, the absorbance at 600 nm was measured.

Electrochemical detection in urine

Human urine (BioreclamationIVT) was centrifuged at 5000 g for 5 min to remove large particulates. *E. coli* and *K. pneumoniae* were diluted to 100 cfu μL^{-1} and spiked in the urine. Samples (200 μL) were introduced at 20 μL min^{-1}. Next, 200 μL of either ampicillin or ciprofloxacin in 1 mM resazurin and LB media were introduced at 20 μL min^{-1}. Air was flushed through the device to form the wells followed by FC-40 (200 μL) (Sigma Aldrich, St. Louis, MO). Thus the total volume of all solutions introduced is 600 μL which requires 30 min to process at 20 μL min^{-1}. The device was incubated in a water bath at 37 °C for 1 hour. 10 minutes were required to scan the leads. Thus the total time for the assay from sample introduction to readout was 1 hour and 40 minutes.

Electrochemical detection in unpurified urine

E. coli were diluted to 100 cfu μL^{-1} and spiked in the unpurified human urine (BioreclamationIVT). The spiked urine (200 μL) was passed through a 10 μm filter to remove large particulates and directly introduced at 20 μL min^{-1} into the chip. Next, 200 μL of either ampicillin or ciprofloxacin in 1 mM resazurin and LB media were introduced at 20 μL min^{-1}. Air was flushed through the device to form the wells followed by FC-40 (Sigma Aldrich, St. Louis, MO). The device was incubated in a water bath at 37 °C.

Electrochemical measurements

Electrochemical measurements were performed using a potentiostat (BASi, West Lafayette, IN) and a three electrode set-up. For off-chip electrochemical experiments, we used a Ag/AgCl reference electrode and a Pt counter electrode. For on-chip experiments, we used the in-well Au reference and counter electrodes. Electrodes were scanned using differential pulse voltammetry.

Results and discussion

Electrochemical detection of viable bacteria

Redox dyes that are reduced by metabolically-active bacteria have been used as optical indicators of bacterial viability in the presence of antibiotics,[19] but have not offered significant improvement in the delivery of rapid profiling results. We hypothesized that resazurin, a commonly-employed reporter used to optically assess cell viability,[19] could be used for electrochemical detection of bacterial antibiotic susceptibility (Fig. 1), and potentially, the sensitivity of this readout method could produce improvements in assay speed. In the presence of an ineffective antibiotic, resistant bacteria will continue to multiply and create a reducing environment which converts resazurin to resorufin. On the other hand, since effective antibiotics hinder bacterial metabolism, they will prevent reduction of the dye. As resazurin and resorufin have different electrochemical signatures,[31,32] using differential pulse voltammetry we can distinguish between the two states of the dye and thus determine whether the bacteria is susceptible (Fig. 1E).

We first characterized the electrochemical profile of resazurin to determine if this reporter group would be suitable for monitoring live bacteria (see Fig. S1 in ESI†). When resazurin is present in aqueous buffer, the initial irreversible two-electron reduction of the dye to resorufin occurs at −0.45 V *vs.* Ag/AgCl. An additional reversible process is observed at −0.6 V that represents the two electron reduction of resorufin to dihydroresorufin.[31,32] In bacterial culture media at 37 °C, the formation of dihydroresorufin occurs at a less negative potential and is visualized as a shoulder on the resazurin reduction peak when differential pulse voltammetry (DPV) is used to monitor the redox reporter. Nonetheless, a significant decrease in the electrochemical signal is observed in the

presence of active bacteria (Fig. S2 in ESI†). We studied the effect of dissolved oxygen on the electrochemical signal and found that it did not significantly affect our measurements (Fig. S3†).

We tested the limit of detection that could be achieved by monitoring the electrochemical signal of resazurin by incubating serial dilutions of *Escherichia coli (E. coli)* with 1 mM resazurin in LB culture media for 5 hours at 37 °C. Fig. 2A shows representative DPV scans and the average peak currents at −0.35 V as a function of bacterial concentration are plotted in Fig. 2B. We obtained a detection limit of 100 cfu μL^{-1}, which is clinically relevant and commonly used as a threshold level for the presence of bacteriuria.[2,33] The peak signals decreases with increasing bacterial concentration, as expected given that viable bacteria convert resazurin to resorufin. As there is significant overlap between peaks I and II, a decrease in the height of peak I causes peak II to decrease as well.

We compared the detection limit of electrochemical and fluorescent detection of bacterial viability using resazurin and found a similar limit of detection of 100 cfu μL^{-1} indicating that electrochemical detection of resazurin is just as sensitive as fluorescent readout (Fig. S4†). The advantage of using electrochemistry is that it does not require complicated or bulky instrumentation for readout and the sensors can be integrated directly into the culture chambers. In the most sensitive fluorescence assays, the assay is performed in a series of nanoliter droplets which require a high-powered fluorescence microscope for sequential readout of the droplets. Using electrochemistry, it is possible to integrate the sensors directly into the nanoliter culture chambers, eliminating the need for sophisticated optical equipment for readout. The electronics required for electrochemical readout can be integrated into a small benchtop or handheld device, limiting the cost and footprint of clinical instrumentation that could be developed with this approach.

Design of a microfabricated device for rapid electrochemical analysis of bacteria

With proof-of-concept in hand demonstrating that bacterial viability could be monitored electrochemically, we explored strategies to decrease the detection time to improve the clinical utility of this assay. We hypothesized that by initially concentrating bacteria in a nanoliter well and subsequently conducting the assay within this small volume, we could reduce the assay time to less than one hour. Integrating electrochemical sensors directly into each of the nanoliter incubation chambers allows rapid and direct readout of the antibiotic susceptibility profile in a small volume without requiring bulky optical instrumentation to sequentially readout thousands of nanoliter droplets.

This approach provides two advantages: concentration and confinement. Concentrating the bacteria inside miniaturized wells increases the local effective concentration of the bacteria. For example, 10 bacteria captured in a 1 nL well is equivalent to 10 000 cfu μL^{-1}, while 10 bacteria captured in 1 μL well gives a concentration of only 10 cfu μL^{-1}. The greater the concentration of bacteria per well, the faster the turnover of resazurin and accumulation of the target redox molecule. As the signal from differential pulse voltammetry is directly proportional to the concentration of the redox molecule, an increase in local concentration of bacteria increases the magnitude of the signal change acquired.

Confinement within a nanoliter volume provides another advantage – as resazurin is reduced, it is prevented from diffusing into bulk solution allowing the reduced form to rapidly accumulate to detectable levels.

A fully integrated device for the concentration of bacteria into an array of independent nanoliter culture chambers was designed with each well equipped with electrodes to electrochemically readout the antibiotic susceptibility profile of the bacteria captured within (Fig. 1B, C). The culture chambers

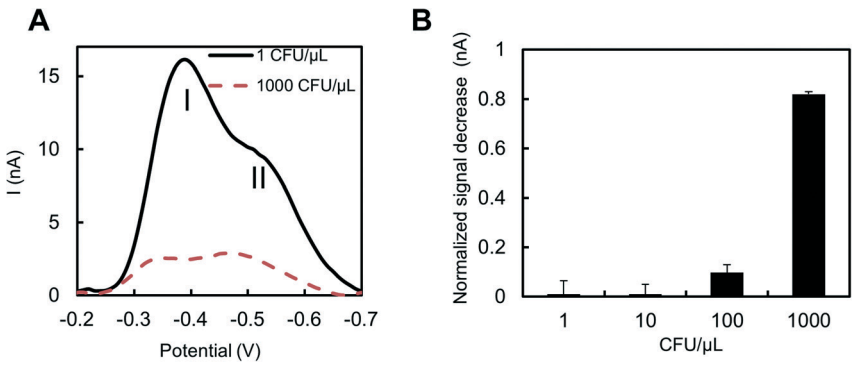

Fig. 2 Electrochemical detection of bacterial metabolism. (A) Representative differential pulse voltammagrams obtained from culturing serial dilutions of *E. coli* for 5 hours with resazurin. Differential pulse voltammograms of resazurin in LB media exhibit two peaks. Peak I corresponds to the conversion of resazurin to resorufin through an irreversible 2-electron process, while peak II corresponds to the reversible reduction of resorufin to dihydroresorufin. Peak I decreases systematically as metabolically active bacteria metabolize resazurin. Electrochemical scans were acquired with respect to the on-chip Au reference electrode which causes the peak current to shift to more negative potentials when compared to the Ag/AgCl reference electrode. (B) Average signal decrease obtained after culturing *E. coli* for 5 hours with resazurin. Data shown represents the average of at least 8 replicates. Error bars represent standard error.

have dimensions of 100 μm × 50 μm × 550 μm, which is equivalent to a volume of 2.75 nL. Each well also contained a filter for bacterial capture. The well array allows for multiple measurements per sample, thereby increasing the accuracy of the device. Typically, 15 measurements are performed per sample.

To fabricate this device, we patterned gold electrodes on a glass substrate which act as the working, counter and reference electrodes (Fig. S5 and S6†). The substrate was passivated with a 2 μm thick SU-8 layer and openings were defined using photolithography to expose the electrodes beneath. A 50 μm thick SU-8 layer was patterned to form the microchannel and culture wells. This was followed by a 2 μm SU-8 layer patterned to define a barrier at the rear of each well. This barrier is used to immobilize a bed of microbeads which act as an in-well bacterial filter (Fig. 1B). Fig. S5† shows optical images of the fabricated device. The working electrodes were electroplated with $HAuCl_4$ to increase the electrode surface area. This increases the magnitude of the acquired signal and thus, the detection sensitivity (Fig. S10A†).

To perform the bacterial detection assay within the nanoliter capture device, bacterial cells are introduced into the main channel, which then flow into the wells (Fig. 1D). The bacteria are prevented by the in-well microbead filters from exiting the wells, while the solution continues to flow. Next, the culture medium, resazurin, and an antibiotic is introduced. An immiscible fluorinated oil, FC-40, is also brought into the device, which displaces the aqueous solution in the main channel. Due to surface tension, a sealed nanoliter plug of culture media remains in each well. The device can then be incubated at 37 °C to allow bacteria to multiply. Antibiotic-susceptible bacteria captured within a well are inhibited from reproducing and do not reduce resazurin while resistant bacteria continue to multiply and reduce the molecule (Fig. 1E). After incubation, electrochemical measurements are recorded using DPV with the on-chip electrodes.

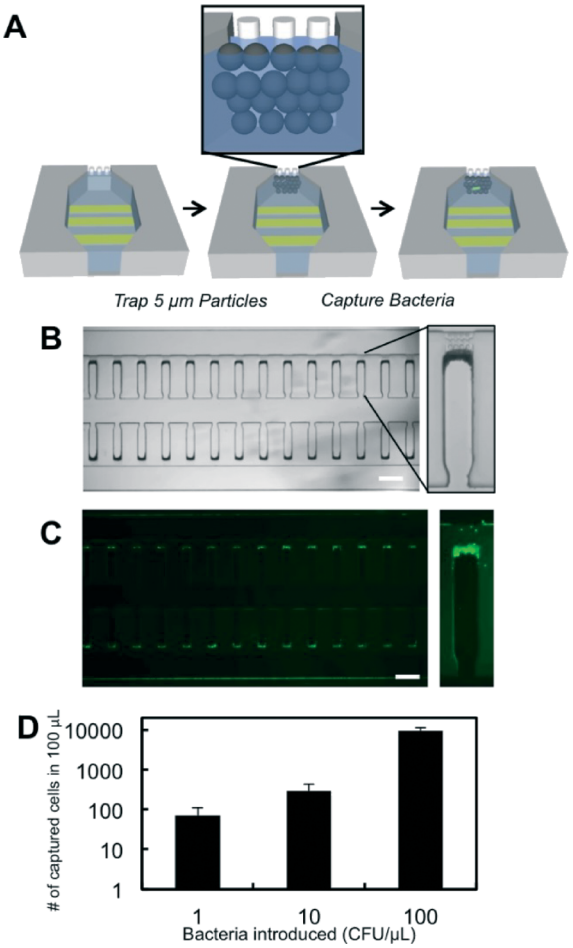

Fig. 3 In-well bacterial capture and analysis. (A) Schematic of in-well bacterial capture. Bacteria are trapped within in-well size-based filters fabricated from a bed of polystyrene beads immobilized within each well. (B) Optical image of an additional filter introduced by microbeads immobilized at a pre-fabricated in-well barrier. (C) *E. coli* expressing GFP are trapped within the wells by the microbead filter. Scale bars represent 300 μm. (D) The number of captured bacteria as a function of the concentration of bacteria introduced. 100 μL of sample was introduced.

Validation of in-well bacterial capture

To concentrate bacteria within the wells, a size-based filtration approach was employed. Within each well, we fabricated an SU-8 barrier with a 2 μm tall gap between the surface and roof of the device. This gap size was the smallest feature that could be reliably patterned. Initial experiments indicated that this gap is too large to trap bacteria efficiently (Fig. S7D†), thus we contemplated other strategies to enhance the levels of bacterial capture. As high-throughput fabrication of submicron features using standard photolithography is challenging, we explored alternative strategies to generate in-well filters with the submicron pore sizes necessary to trap bacteria. While too large to trap bacteria directly, this in-well 2 μm gap is sufficiently small to trap 5 μm diameter microbeads. Microbeads trapped by the barrier self-assemble into an array that acts as a bacterial filter (Fig. 3A, B).[34] The submicron pores between beads prevent the passage of bacteria allowing bacteria introduced into the device to concentrate within the wells.

To further characterize the filters, we measured the filter stability (Fig. S8†). We found that the filter bed was stable for at least 1 hour after stopping the flow, which is the time required for incubation. Next, we estimated the pore size assuming hexagonal close packing of the spherical microbeads. We calculated a minimum pore size of 0.77 μm, which is sufficiently small to trap a bacterium. Using optical microscopy, we confirmed the validity of these calculations (Fig. S7A–S7C†). These calculations assume perfect hexagonal close packing, but in reality the beads will assemble in a geometry resembling random close packing which causes a distribution in pore sizes, but does not change the diameter of the smallest pores.

We challenged the system with *E. coli* expressing green fluorescent protein (GFP) and visualized the presence of

captured bacteria using fluorescence microscopy (Fig. 3C). Our initial experiments indicated that bacteria were reproducibly captured within each well. To quantitate the capture efficiency of our device, we introduced serial dilutions of a 100 μL volume of GFP *E. coli* at a flow rate of 10 μL min^{-1}. After capture, bacteria were introduced onto agar plates and the *E. coli* colonies were counted after incubating the plates overnight. Fig. 3D shows the capture efficiency as a function of concentration. Our results indicate this device achieves ~80% capture at concentrations as low as 1 cfu μL^{-1}. As the microbeads assemble randomly, there is a distribution of pore sizes, which allows some bacteria to escape to the filter. As expected, the capture efficiency decreases as a function of flow rate (Fig. S7E†). In the case of the 100 cfu μL^{-1} sample, given that there are 72 wells per device, each well captured on average 120 bacteria. Considering that each well has a volume of 2.5 nL, this represents an effective concentration of approximately 50 000 cfu μL^{-1}. This represents a 500-fold concentration enhancement above the initial concentration of 100 cfu μL^{-1}.

On-chip detection of viable bacteria

With effective capture demonstrated, we tested the ability of our electrochemical assay to detect viable bacteria captured within the wells. We challenged the device with *E. coli* at 100 cfu μL^{-1}, a clinically relevant concentration in urinary tract infections.[2] This concentration corresponds to over 100 bacteria per well. We studied the time dependence of the signal to determine the minimum time necessary to detect a clinically relevant concentration of viable bacteria. As shown in Fig. 4, we achieved successful detection of metabolically-active bacteria within 30 minutes. In control experiments, we did not observe a statistically significant signal change after 1 hour with a blank culture media sample without spiked bacteria (Fig. 4). These results represent a greater than 5-fold reduction in incubation time over the previous record of 2.8 h.[20] We observed a small signal increase in the case of blank media which may be due to small chip-to-chip variations. We observe some signal decrease in the case of blank urine and unpurified urine which could be attributed to surface fouling of the electrode as well.

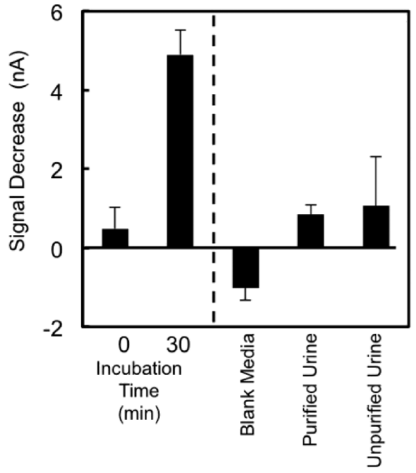

Fig. 4 In-well electrochemical measurement of bacterial viability. Electrochemical signal generated by resazurin decreases as a function of incubation time for *E. coli* at 100 cfu μL^{-1}. Viable *E. coli* are detected within 30 minutes. No positive signal change is observed after a 60 minute incubation with a blank culture media and both purified and unpurified urine. The signal decrease is calculated by subtracting the acquired peak current from the maximum peak current of 11 nA.

Detection of antibiotic susceptibility in complex matrices

After demonstrating detection of viable bacteria, we assessed the suitability of the device to rapidly determine the antibiotic resistance profile of bacteria in undiluted urine. To better simulate a clinical sample, we chose to test uropathogenic strains of *E. coli* (UPEC) and *Klebsiella pneumoniae (K. pneumoniae)*, two of the most common pathogens implicated in urinary tract infections.[2] The *K. pneumoniae* strain was isolated from the urine of an infected patient and produces extended spectrum β-lactamase enzymes which confer resistance to a wide variety of β-lactam antibiotics.[2] We chose to test for susceptibility to two commonly used antibiotics to treat urinary tract infections – ampicillin, a β-lactam antibiotic, and ciprofloxacin, a fluoroquinolone.[35]

In order to determine a suitable incubation period, we measured the minimum time required for these antibiotics to affect bacterial metabolic activity (Fig. S11†). We found that ciprofloxacin inhibited the metabolic activity of *K. pneumoniae* within 30 minutes indicating that a 1 hour incubation period is sufficiently long. We studied the effect of surface fouling induced by incubating the devices with LB media (Fig. S10B†) for 1 hour. We noticed only a small change in the acquired signals before and after incubation indicating that fouling could be attributed to approximately a 15% signal change which is acceptable as these devices are not designed to be reused.

E. coli (UPEC) and *K. pneumoniae* present at 100 cfu μL^{-1} in undiluted urine were introduced into the device. After capture, we introduced a culture medium, resazurin, and either ampicillin or ciprofloxacin. Fig. 5C and D show the signal obtained as a function of antibiotic concentration after a 1 hour incubation. No signal change was observed with a blank sample of urine (Fig. 4).

For the *E. coli* strain, the signal decrease is low for all ciprofloxacin concentrations, indicating the bacteria are susceptible to the antibiotic at concentrations above 1 μg mL^{-1} (Fig. 5C). The signal decrease is reduced with ampicillin concentration indicating susceptibility at concentrations between 10 and 100 μg mL^{-1}. We confirmed our results using a standard microdilution assay with a 24 hour incubation (Fig. 5A). We found that the minimum antibiotic concentration that inhibits 90% of bacterial growth (MIC_{90}) is 16 μg mL^{-1} for ampicillin and less than 0.1 μg mL^{-1} for ciprofloxacin.

For *K. pneumoniae*, the signal is approximately constant with increasing ampicillin concentration, indicating that

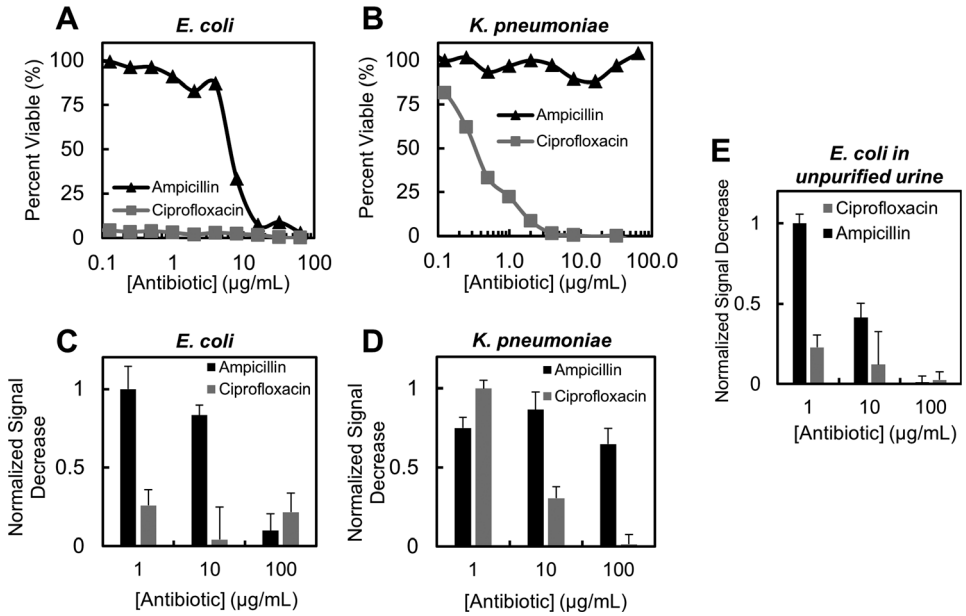

Fig. 5 Electrochemical phenotypic testing of antibiotic-resistant bacteria. (A) and (B) Determination of the antibiotic resistance profile of *E. coli* and *K. pneumoniae* to ampicillin and ciprofloxacin using a standard microdilution assay with a 24 hour incubation. (C) and (D) Electrochemical determination of the antibiotic susceptibility of *E. coli* and *K. pneumoniae* on-chip after incubating with different levels of antibiotic at 37 °C for 1 hour. (E) On-chip electrochemical determination of the antibiotic susceptibility of *E. coli* in unpurified urine after incubating with different levels of antibiotic at 37 °C for 1 hour. Currents are normalized to the maximum value. Error bars represent standard error.

bacterial viability is not affected by the ampicillin dose – the hallmark of resistance. As this strain produces a beta lactamase, resistance to ampicillin, a beta-lactam antibiotic is expected. In contrast, we observe a concentration-dependent signal with ciprofloxacin, indicating that bacterial viability is reduced by increasing ciprofloxacin concentration. This indicates that this strain is susceptible to ciprofloxacin and is inhibited at concentrations between 1 and 10 μg mL^{-1} (Fig. 5D). Using a standard microdilution assay we found the *K. pneumoniae* were indeed resistant to ampicillin but susceptible to ciprofloxacin (Fig. 5B). The MIC_{90} is 2 μg mL^{-1} for ciprofloxacin and greater than 100 μg mL^{-1} for ampicillin. For both strains, our results on-chip show good agreement with the MIC determined using this gold standard method which required incubation times over 20 times longer than the on-chip assay. We found good correlation between the on-chip susceptibility assay and standard assays with r^2 values of 0.81 and 0.82 for *E. coli* and *K. pneumoniae* respectively (Fig. S12†). This discrepancy may be attributable to the different detection methodologies and incubation periods used when comparing the two methods.

Detection of antibiotic susceptibility in unpurified urine

Next, we performed a series of experiments to determine the antibiotic susceptibility of bacteria in unpurified urine (Fig. 5E). *E. coli* were spiked directly into undiluted and unpurified urine at 100 cfu μL^{-1}. The sample was passed through a 10 μm filter that removed large particulates while allowing bacteria to pass (Fig. S13†). The filtrate was introduced into the device and tested against ampicillin and ciprofloxacin by incubating at 37 °C for 1 hour. We found that the *E. coli* were susceptible to ciprofloxacin at concentrations 1 μg mL^{-1} and above and susceptible to ampicillin at a concentration between 10 and 100 μg mL^{-1}. We found no appreciable signal change when using a blank control sample of unpurified urine (Fig. 4). These results agree with the standard microdilution antibiotic susceptibility assay indicating that the device can be challenged with unpurified urine samples using a simple inline pre-filtration sample processing step. Representative electrochemical scans acquired on-chip are included in Fig. S14.†

This device offers the fastest reported detection of antibiotic susceptibility at clinically relevant concentrations directly from unpurified urine. The rapid turnaround time is facilitated by concentrating the bacteria in a nanoliter volume which drastically increases the local effective concentration of bacteria. The turnaround time is further reduced by incubating the bacteria in isolated nanoliter compartments which allows the reduced form of resazurin to rapidly accumulate to detectable levels by confining diffusion. A third advantage of this approach is that it is purely electronic, which facilitates the development of antibiotic susceptibility tests at the point-of-care by eliminating the need for expensive and bulky optical equipment.

In a clinical setting, this device could serve as a rapid alternative to standard susceptibility tests to provide results with a 1 hour incubation after initial culture-based identification of the bacteria. Currently, standard antibiotic susceptibility tests require an additional 18–24 hours after the initial culture step.

The rapid-response device could also be used in conjunction with standard culture-based antibiotic susceptibility tests to provide point-of-care susceptibility results directly from undiluted urine with a 1 hour incubation period. This would thereby permit the rapid administration of an effective antibiotic in the interim until the results of standard antibiotic susceptibility tests are available 2–3 days later at which point the therapy could be refined. This would allow doctors to administer a targeted antibiotic almost immediately, which would improve patient outcomes and curb the rise of antibiotic resistance by decreasing the use of broad spectrum antibiotics. In infections which lead to urosepsis, the most severe UTIs, this device would have the greatest clinical utility as these infections require immediate administration of effective antibiotics.[4]

When challenged with a sample containing a single bacterial strain, our device accurately and rapidly determines the susceptibility to various antibiotics. To enable accurate detection in the case of multiple infecting species (polymicrobial infections are present in only 5%–11% of individuals with urosepsis[36]), the multiple nanoliter chambers employed herein could be devoted to multiplexed combinations of bacteria combined with local metabolic sensing.

Conclusions

Using an electrochemical approach capable of detecting metabolically active bacteria, we have demonstrated the detection of live bacteria using a short 30 minute incubation period. By concentrating and analysing the bacteria within miniaturized compartments, the time required to detect viable bacteria is drastically reduced. We utilize this assay to monitor bacterial metabolism in response to antibiotics to rapidly readout the antibiotic susceptibility profile. This approach could allow for rapid administration of antibiotics before the results of standard culture-based susceptibility testing are available.

Acknowledgements

The authors wish to thank the Natural Sciences and Engineering Research Council for funding in support of this work (Discovery Grant to S. O. K.). J. D. B. acknowledges support from the Ontario Graduate Scholarship.

Notes and references

1 S. B. Levy and B. Marshall, *Nat. Med.*, 2004, **10**, S122–S129.
2 B. Foxman, *Nat. Rev. Urol.*, 2010, **7**, 653–660.
3 M. A. Pfaller and R. N. Jones, *Arch. Pathol. Lab. Med.*, 2006, **130**, 767–778.
4 F. M. E. Wagenlehner, A. Pilatz and W. Weidner, *Int. J. Antimicrob. Agents*, 2011, **38**, 51–57.
5 W. J. McIsaac and C. L. Hunchak, *Med. Decis. Making*, 2011, **31**, 405–411.
6 V. Perreten, L. Vorlet-fawer, P. Slickers, R. Ehricht, P. Kuhnert and J. Frey, *J. Clin. Microbiol.*, 2005, **43**, 2291.
7 B. Strommenger, C. Kettlitz, G. Werner and W. Witte, *J. Clin. Microbiol.*, 2003, **41**, 4089.
8 S. Shenai, F. Krapp, J. Allen, R. Tahirli, R. Blakemore, R. Rustomjee, A. Milovic, M. Jones, S. M. O. Brien, D. H. Persing, S. Ruesch-gerdes, E. Gotuzzo, C. Rodrigues, D. Alland and M. D. Perkins, *N. Engl. J. Med.*, 2010, **363**, 1005–1015.
9 K. E. Mach, R. Mohan, E. J. Baron, M.-C. Shih, V. Gau, P. K. Wong and J. C. Liao, *J. Urol.*, 2011, **185**, 148–153.
10 M. C. Roberts, S. Schwarz and H. J. M. Aarts, *Front. Microbiol.*, 2012, **3**, 384.
11 G. Longo, L. Alonso-Sarduy, L. M. Rio, A. Bizzini, A. Trampuz, J. Notz, G. Dietler and S. Kasas, *Nat. Nanotechnol.*, 2013, **8**, 522–526.
12 T. S. Mann and S. R. Mikkelsen, *Anal. Chem.*, 2008, **80**, 843–848.
13 Y. Lu, J. Gao, D. D. Zhang, V. Gau, J. C. Liao and P. K. Wong, *Anal. Chem.*, 2013, **85**, 3971–3976.
14 P. Ertl, B. Unterladstaetter, K. Bayer and S. R. Mikkelsen, *Anal. Chem.*, 2000, **72**, 4949–4956.
15 P. Ertl, M. Wagner, E. Corton and S. R. Mikkelsen, *Biosens. Bioelectron.*, 2003, **18**, 907–916.
16 K. Chotinantakul, W. Suginta and A. Schulte, *Anal. Chem.*, 2014, **86**, 10315–10322.
17 B. Li, Y. Qiu, A. Glidle, D. McIlvenna, Q. Luo, J. Cooper, H.-C. Shi and H. Yin, *Anal. Chem.*, 2014, **86**, 3131–3137.
18 M. W. Kadlec, D. You, J. C. Liao and P. K. Wong, *J. Lab. Autom.*, 2013, **19**, 258–266.
19 J. Palomino, A. Martin, M. Camacho, H. Guerra, J. Swings and F. Portaels, *Antimicrob. Agents Chemother.*, 2002, **42**, 2720–2722.
20 J. Q. Boedicker, L. Li, T. R. Kline and R. F. Ismagilov, *Lab Chip*, 2008, **8**, 1265–1272.
21 K. Churski, T. S. Kaminski, S. Jakiela, W. Kamysz, W. Baranska-Rybak, D. B. Weibel and P. Garstecki, *Lab Chip*, 2012, **12**, 1629–1637.
22 F. Deiss, M. E. Funes-Huacca, J. Bal, K. F. Tjhung and R. Derda, *Lab Chip*, 2014, **14**, 167–171.
23 N. J. Cira, J. Y. Ho, M. E. Dueck and D. B. Weibel, *Lab Chip*, 2012, **12**, 1052–1059.
24 C. H. Chen, Y. Lu, M. L. Y. Sin, K. E. Mach, D. D. Zhang, V. Gau, J. C. Liao and P. K. Wong, *Anal. Chem.*, 2010, **82**, 1012–1019.
25 D.-K. Kang, M. M. Ali, K. Zhang, S. S. Huang, E. Peterson, M. A. Digman, E. Gratton and W. Zhao, *Nat. Commun.*, 2014, **5**, 5427.
26 M. Safavieh, M. U. Ahmed, M. Tolba and M. Zourob, *Biosens. Bioelectron.*, 2012, **31**, 523–528.
27 M. Varshney, Y. Li, B. Srinivasan and S. Tung, *Sens. Actuators, B*, 2007, **128**, 99–107.
28 K. Hsieh, A. S. Patterson, B. S. Ferguson, K. W. Plaxco and H. T. Soh, *Angew. Chem., Int. Ed.*, 2012, **51**, 4896–4900.
29 A. S. Patterson, K. Hsieh, H. T. Soh and K. W. Plaxco, *Trends Biotechnol.*, 2013, **31**, 704–712.
30 L. Soleymani, Z. Fang, B. Lam, X. Bin, E. Vasilyeva, A. Ross, E. H. Sargent and S. O. Kelley, *ACS Nano*, 2011, **5**, 3360.
31 S. Çakir and E. Y. Arslan, *Chem. Pap.*, 2010, **64**, 386–394.

32 S. Khazalpour and D. Nematollahi, *RSC Adv.*, 2014, **4**, 8431.
33 J. W. Warren, E. Abrutyn, J. R. Hebel, J. R. Johnson, A. J. Schaeffer and W. E. Stamm, *Clin. Infect. Dis.*, 1998, **29**, 745–758.
34 N. Bao, B. Jagadeesan, A. K. Bhunia, Y. Yao and C. Lu, *J. Chromatogr. A*, 2008, **1181**, 153–158.
35 K. Gupta, T. M. Hooton, K. G. Naber, B. Wullt, R. Colgan, L. G. Miller, G. J. Moran, L. E. Nicolle, R. Raz, A. J. Schaeffer and D. E. Soper, *Clin. Infect. Dis.*, 2011, **52**, e103–e120.
36 O. Braissant, G. Müller, A. Egli, A. Widmer, R. Frei, A. Halla, D. Wirz, T. C. Gasser, A. Bachmann, F. Wagenlehner and G. Bonkat, *J. Clin. Microbiol.*, 2014, **52**, 624–626.