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A Microfluidic Device for Nucleic Acid-Based Analysis of *Helicobacter pylori* and Antibiotic Susceptibility Testing

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Abstract

Rapid diagnosis of bacterial infections enables earlier implementation of appropriate and effective treatment regimens, therefore improving patient outcomes. We have previously demonstrated a microfluidic device for nucleic acid-based analysis of *Helicobacter pylori*, which can cause peptic ulcers and increase the risk of stomach cancer. One of the major challenges to overcome has been the use of challenging real-world samples, e.g. stool, and urine allowing use at the point-of-care. Here we present results for bacterial identification as well as initial studies on the use of screen-printed electrodes (SPE) for miniaturised electrochemical detection for determining antibiotic susceptibility of pathogenic *Escherichia coli*. Resazurin was used as an indicator of bacterial viability, with reduction of resazurin demonstrating continued cell growth. Differential pulse voltammetry was used to measure potential reduction and showed measurement at -0.58 V or -0.38 V to be the most discriminatory. A fixed voltage of -0.58 V was then used to monitor current changes as a function of increasing gentamycin (antibiotic) concentration, with the results showing a significant reduction in current with increasing amounts of gentamycin (ANOVA, $p < 0.001$), within 90 min. The ability to integrate diagnosis with antibiotic susceptibility testing would allow administration of timely and specific treatment to patients with serious infections in low-resource settings.

Keywords

Microfluidics • Bacterial infection • Antibiotic susceptibility

1 Introduction

Bacterial infections affect hundreds of thousands of people annually worldwide. Rapid diagnosis of bacterial infections enables earlier implementation of appropriate and effective treatment regimens, therefore improving patient outcomes. Patient samples are preferentially collected using non-invasive methods, i.e. urine or stool samples, for both ease of collection and improved patient compliance. However, both sample types present challenges for analysis in terms of low target analyte concentrations and heterogeneity. Bacterial strain identification is important as different strains can have varying pathogenicity, for example, the phenotype of *Helicobacter pylori* (*H. pylori*) that expresses cytotoxin-associated protein (CagA) causes a higher degree of acute inflammation and is three times more likely to lead to gastric carcinogenesis than *H. pylori* strains which do not express CagA [1]. We have previously demonstrated a microfluidic device for nucleic acid-based analysis of *H. pylori*, from clinical stool samples, which identified the bacterial infection and whether or not it expressed the pathogenic CagA [2].

The growing emergence of antibiotic resistance is a global concern and has been identified as a high priority for the World Health Organisation [3]. Therefore the ability to not only identify the type of infection but also which antibiotics can be prescribed to successfully treat the infection (i.e. antibiotic susceptibility) is crucial to improve clinical outcomes. The use of microfluidics to integrate these steps together offers the potential to create cost-effective diagnostics for point-of-care applications.

Resazurin has been commonly used as a redox indicator to measure the metabolic rate of bacteria, which can be indicative of antibiotic resistance/susceptibility. In aqueous buffer solution, resazurin (blue) undergoes an irreversible two-electron reduction to resorufin (pink) due to the elimination of oxygen from the dye molecule. An additional two electron reversible reduction process to convert resorufin to

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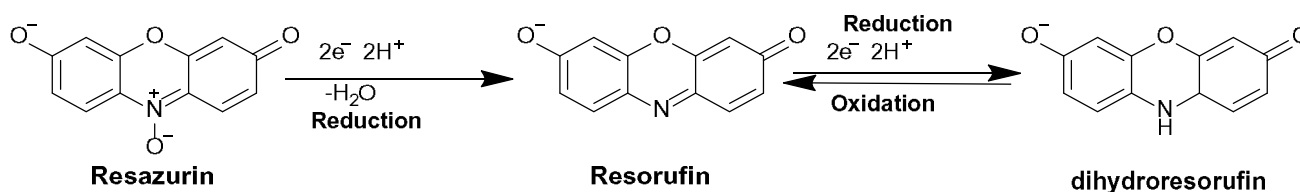


Fig. 1 Reduction mechanism of Resazurin in aqueous buffer medium [4]

dihydroresorufin (colourless) can also occur (Fig. 1). Traditionally reduction of resazurin has been monitored using colourimetric or fluorescence assays which vary in sensitivity.

Here we present work on bacterial identification and initial studies on the use of screen-printed electrodes (SPE) for miniaturised electrochemical detection for determining antibiotic susceptibility of pathogenic bacteria.

2 Experimental

Solidworks[®] was used to produce 3D printed moulds. Polydimethylsiloxane (PDMS) (Sylgard 182, Dow Corning) was then cast into the moulds to create the microfluidic devices (Fig. 2).

Electrochemical measurements were performed using a potentiostat (EmStat—PalmSens, NL) and a three-electrode setup. For off-chip electrochemical experiments, CH gold electrodes (diameter 2 mm) were used with Pt counter and Ag/AgCl reference electrodes. For on-chip electrochemical experiments, screen printed carbon working electrodes (diameter 1.5 mm) were used with a screen printed Pt counter and Ag/AgCl reference electrode (Fig. 3). Both working electrodes were scanned using differential pulse voltammetry (DPV) Resazurin (Sigma-Aldrich, UK) was used as the redox indicator.

Bacterial cultures (*Escherichia coli* and *Staphylococcus aureus*) used for control experiments were grown up in nutrient broth (Fisher Scientific, UK) overnight at 37 °C. Gentamycin (Merck, UK) was used as an example antibiotic. Clinical stool samples were obtained from NHS Chesterfield Laboratories; samples were fully anonymized and were

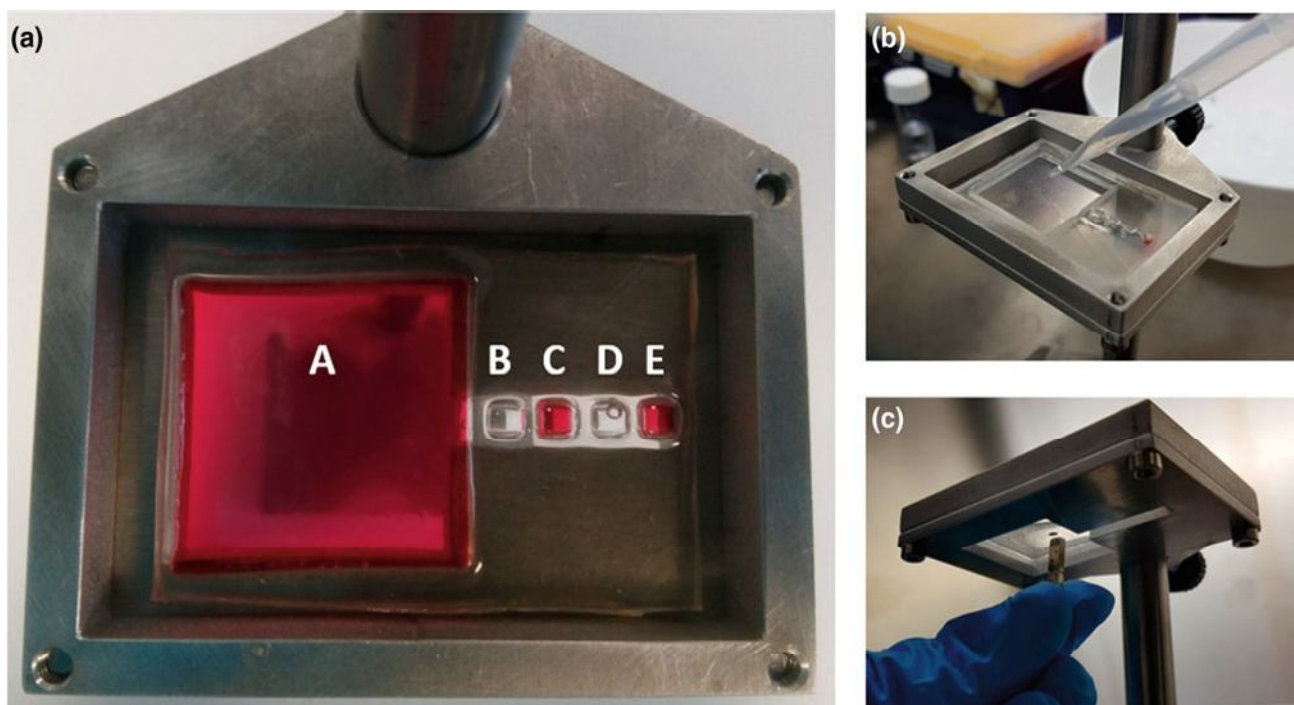


Fig. 2 a Overview of the design of the microfluidic device for bacterial identification loaded with food dyes to clearly indicate (A) sample chamber (pre-loaded with superparamagnetic particles (PMPs) and powdered guanidine hydrochloride (GuHCl), (B & D)

mineral oil, (C) GuHCl wash and (E) elution/amplification chambers; b sample addition and c movement of PMPs using an external NdFeB magnet from chamber A to E

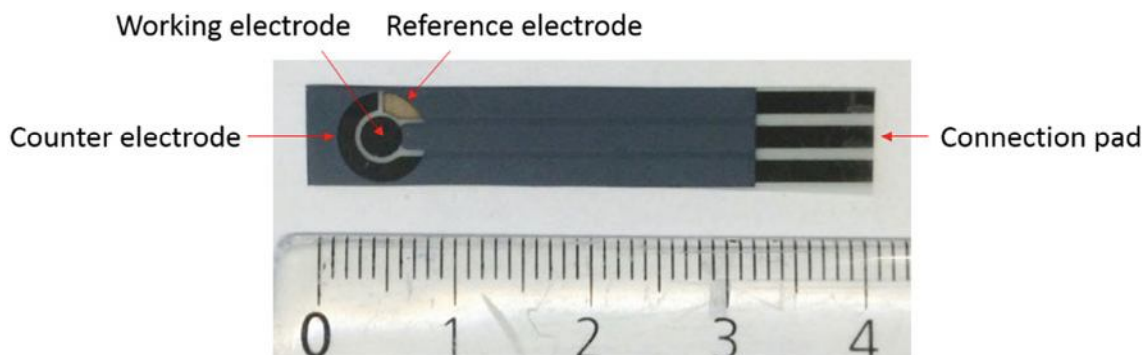


Fig. 3 Screen-printed electrode

selected on the basis that they had previously been tested for *H. pylori* during routine clinical testing.

3 Results and Discussion

Chemical lysis of bacterial cells was achieved on-chip using guanidine hydrochloride powder stored in the sample chamber, and reconstituted upon sample addition. Lysis efficiencies of 80.2 and 69.3%, respectively, for *E. coli* (Gram negative) and *S. aureus* (Gram positive) were demonstrated. DNA extraction efficiencies, using *E. coli* as a model organism, were shown to be greater (~75%) when smaller amounts of DNA were present, which is ideal for dealing with low levels of infection in clinical specimens. The use of multiple wash chambers (Fig. 2, chambers B–D) was shown to be necessary when analysing stool samples to provide DNA of sufficient purity for amplification of target markers: Ure C (for identification of *H. pylori*) and CagA (indicator of severe pathogenic phenotype) [2].

As resazurin and resorufin have different electrochemical signals in aqueous buffer solution, by using differential pulse voltammetry it is possible to identify the oxidation/reduction states of resazurin and thus specify whether the target bacteria is susceptible or not [5]. Figure 4, shows the differential pulse voltammogram recorded during the reduction of resazurin in the three electrode configuration. It is observed that the two electron reduction of resazurin to resorufin occurs at -0.38 V (Peak I). An additional two electron reversible reduction process is observed at -0.58 V (Peak II) that corresponds to formation of dihydroresorufin in the same aqueous medium.

A fixed voltage of -0.58 V was then used to monitor current changes as a function of increasing gentamycin concentration (10, 20, 30, 40 and 50 mg/mL), with the results showing a significant reduction in current with increasing amounts of gentamycin (ANOVA, $p < 0.001$), within 90 min (Fig. 5). In Fig. 5, it was observed that, both

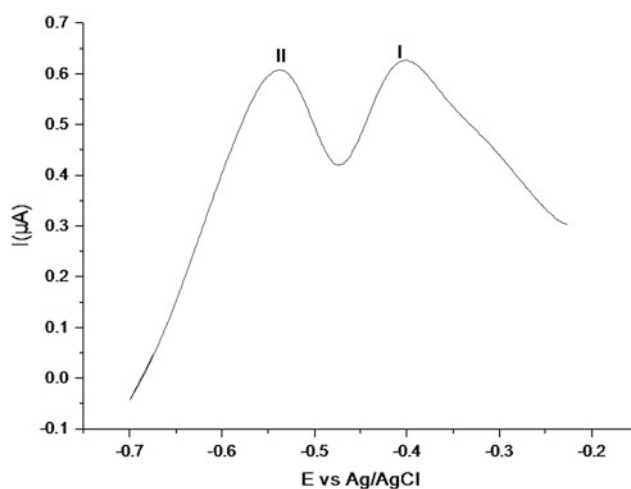


Fig. 4 Representative differential pulse voltammogram recorded during the reduction of buffered resazurin solution (1 mM)

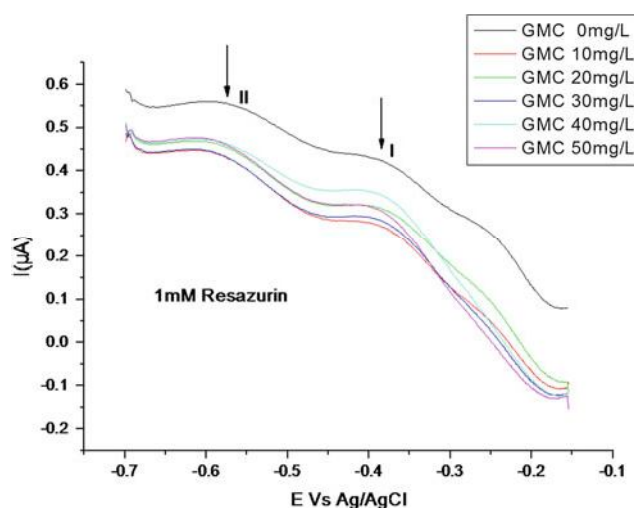


Fig. 5 Representative differential pulse voltammograms obtained from cultured *E. coli* for 80 min with resazurin and gentamycin (GMC)

peaks I and II decreased systematically as metabolism was affected by the antibiotic, indicating antibiotic susceptibility.

4 Conclusions

The ability to integrate diagnosis with rapid antibiotic susceptibility testing would allow administration of timely and specific treatment to patients with serious infections in low-resource settings. The work presented here shows how PDMS microfluidic devices can be used for identification of bacterial infections (including phenotypic indications) using genetic testing and also monitoring of the metabolic state of the bacteria using SPEs within 90 min.

Conflict of Interest The authors declare no conflict of interest.

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