RAPID, LOW-COST DETECTION OF PATHOGENIC BACTERIA FOR POINT-OF-CARE DIAGNOSTICS

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ABSTRACT

We demonstrate a novel assay for rapid, low-cost detection of pathogenic bacteria for a microfluidic point-of-care (POC) diagnostic using silver enhancement. The described method allows bacteria to be visually observed without the need of fluorescence or expensive imaging equipment. A proof-of-concept sandwich assay is performed to detect and enumerate individual E. coli O157:H7. The total time for the assay is less than two hours. This assay allows individual cells to be counted and has potential for high-throughput screening of samples for bacteria contamination, while offering a theoretical sensitivity of single cell detection.

KEYWORDS: Point-of-care, Diagnostic, Bacteria, Silver, Assay, Bacteriophage, Hospital Acquired Infection

INTRODUCTION

Hospital acquired (HA) infections of antibiotic resistant bacteria are a growing problem worldwide, costing billions of dollars in treatment and resulting in tens of thousands of deaths in North America alone [1]. Current methods of diagnosing HA infections involve obtaining a sample, culturing the bacteria, and detection by PCR or agar plating with antibiotics. While these methods are fairly sensitive and accurate, they are time consuming (1-3 days) and have limited throughput. Screening for HA infections is therefore priority based (patients scheduled for upcoming surgery) and does not focus on reducing the spread of infection by those unknowingly colonized. To address this problem, we are developing a rapid, low-cost, POC biosensor to detect pathogenic bacteria. A proof-of-concept assay that can be incorporated into this biosensor is demonstrated here using E. coli K12 and attenuated O157:H7 (a common pathogenic strain).

THEORY

A rapid, low-cost sandwich assay based on surface expressed proteins was developed to detect bacteria. In the biosensor, immobilized antibodies (bacteriophage were also investigated as potential binders) against a specific strain of E. coli capture whole cells from the sample (Figure 1, Step 1). Next, a biotinylated detection antibody coats the outer surface of the bacteria (Figure 1, Step 2). This is followed by streptavidin conjugated to gold nanoparticles that bind to the biotinylated antibodies (Figure 1, Step 3). Finally, the gold nanoparticles catalyze the reduction of silver from silver enhancement reagents (Figure 1, Step 4) commonly used for histological staining of tissue samples; this forms a coating of silver on individual cells making them easily visible by dark field microscopy as bright particles, or by bright field microscopy as dark spots, eliminating the need for fluorescence microscopy or expensive imaging equipment. This simple assay is ideally suited for microfluidic devices capable of high-throughput screening of samples for a variety of applications, and offers a general approach for the detection of bacteria contamination.

Figure 1: Complete silver enhancement assay using antibodies. Step 1 - Capture of E. coli from sample. Step 2 - Biotinylated detection antibodies. Step 3 - Streptavidin nanogold. Step 4 - Gold nanoparticles catalyze silver reduction.
EXPERIMENTAL

T4 bacteriophage specific for *E. coli* K12 and antibodies specific for *E. coli* O157:H7 were examined as potential binders for bacteria capture. Conditions for binder immobilization were optimized using a microarray format (Figure 2) with hygroscopic buffers (50% glycerol vs. 2M betaine + 25% 1,3-butanediol) at varied pH (7.5, 8.0, 8.5, 9.0) on different surfaces (Xenobind®, Poly-L-lysine, APTES/Glutaraldehyde, Epoxy). Spotted solutions were allowed to bind overnight, followed by blocking the surface with 2% BSA in PBS for 3 hours.

Optimal immobilization conditions were identified by the maximum density of captured bacteria. For T4 bacteriophage, *E. coli* K12 was transfected with a plasmid for green fluorescent protein (GFP) expression. For antibodies, *E. coli* O157:H7 were labeled with STYO9 nucleic acid cell stain. *E. coli* samples were incubated for 30 minutes prior to washing (x3) with PBST (0.05% Tween-20).

Antibodies against *E. coli* O157:H7 were used for completing the proposed silver enhanced assay. Capture antibodies (100 µg/ml) were incubated overnight on Xenobind® slides in 16 well gaskets. 10⁶ cfu/ml of unlabeled *E. coli* O157:H7 in PBS was incubated for 30 minutes followed by washing (x3) with DI water (wash buffers interfere with the reduction of silver). Detection antibodies (100 µg/ml) were incubated for 30 minutes, followed by streptavidin nanogold for 30 minutes. Silver enhancement reagents (Sigma-Aldrich) were allowed to develop for 5 minutes, followed by washing with DI water (x3). Silver amplified bacteria were then examined under dark field; enumeration of bacteria was completed in ImageJ.

RESULTS AND DISCUSSION

The optimal condition for phage immobilization was found to be 10¹² pfu/ml on reactive aldehyde surfaces (Xenobind®) at high pH (9.0) with 50% glycerol printing buffer, yielding a density of roughly 2.5×10⁴ bacteria/mm² (Figure 3 A). It is presumed the glycerol buffer is superior to the 2M betaine + 25% 1,3-butanediol buffer since the higher viscosity better preserves the fragile tail fibers of the phage that are responsible for binding. Higher pH appears to improve immobilization, likely due to increased reactivity for covalent bonding. The capture ability of phage doesn’t appear to be negatively impacted in the pH range examined (Figure 3 B).

Antibodies against *E. coli* O157:H7 were also found to capture cells with high density and reduced background when compared to phage (Figure 4 A), likely due to a lower sensitivity to shear stress during washing. Capture (O157:H7 specific) and detection (biotinylated, O serotype specific) antibodies were therefore chosen to complete the silver assay; the negative control (blocked with 2% BSA) shows no observable bacteria (Figure 4 B vs. C).
Figure 4: Antibody capture and detection of E. coli O157:H7. A) Fluorescently stained E. coli O157:H7 captured by spotted antibodies with low background, scale bar shows 100 µm. B) Dark field image of silver enhanced E. coli O157:H7 vs. C) the negative control blocked with 2% BSA. Scale bars (B,C) show 10 µm.

Individual cells become easily visible as bright spots under dark field following the silver enhancement assay. On flat glass surfaces, a sensitivity of roughly $10^7$ cfu/ml is achieved (Figure 5). Future work will improve sensitivity by using a packed bed of microbeads to increase surface area interactions for bacterial capture; similar approaches with micropost arrays coated with EpCAM antibody have demonstrated highly effective capture of rare circulating tumor cells (CTCs) from whole blood [2].

Figure 5: Standard curve for E. coli O157:H7 detection using the silver amplification assay on flat glass.

CONCLUSION
We present a novel assay for whole cell bacteria detection, ideal for rapid POC diagnostics in hospital settings. Bacteria detected by silver enhancement are easily counted, offering potential for high sensitivity. Since fluorescence and high magnification microscopes are not needed, inexpensive imaging equipment can be used to detect contamination. This assay is significantly faster than conventional agar plating and PCR, and is easily integrated into low-cost, disposable biosensors that may help prevent the spread of HA infections.

ACKNOWLEDGEMENTS
We acknowledge funding from the Biomedical Engineering (BME) Department, McGill University, and the NSERC-CREATE Integrated Sensors and Systems (ISS) training program.

REFERENCES

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