BACTERIA ISOLATION FROM WHOLE BLOOD FOR SEPSIS DIAGNOSTICS

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ABSTRACT

Rapid and reliable detection of bloodstream infections would gain a lot from improved and straightforward isolation of highly purified bacteria from whole blood. Here, we report a microfluidics-based sample preparation strategy to continuously isolate microorganisms from whole blood for downstream analysis. The continuous-flow method takes advantage of the fact that bacteria cells have rigid cell wall enables selective and complete blood cell lysis while ~100% of bacteria are readily recovered. The method as a sample preparation unit offers opportunities to develop molecular based POC for sepsis diagnostics.

KEYWORDS: Bacteria isolation, Infectious disease, Selective cell lysis, Sepsis

INTRODUCTION

In the EU alone, sepsis claims ~146,000 lives/year and is responsible for 7.6 billion EUR/year health care costs. Current standard culture-based diagnosis of sepsis takes 3-7 days to identify the pathogen species and its associated antibiotic susceptibility. Therefore, initial antibiotic treatment is usually empiric and associated with significant mortality. In addition, broad use of antibiotics has resulted in new problems, the emergence of antibiotic resistant microorganisms. One of the causes of antibiotic resistance is the empirical use of drugs. In primary care, prescription of antibiotics is based on the clinical picture only, whereas in hospitals some information may be available on the pathogen by a gram stain for example or another rapid test. However, precise data on the aetiology of the infection and information about susceptibility to antibiotics still takes at least two days. Targeted antibiotic prescription is hampered by the lack of rapid diagnostic procedures available to the clinician.

During the past decades the microbiological diagnostic potential has significantly increased, mainly through implementing amplification methods of DNA and RNA, which are now generally accepted as important and universal diagnostic targets [1]. Molecular diagnostics have also become an important tool in the diagnosis of infectious diseases [2]. Moreover, the ongoing search for new nucleic acid based techniques that can be applied to the diagnosis of infectious diseases has driven technology forward. Despite recent developments in PCR based assays, several challenges, such as contamination from blood cells limiting sensitivity combined with complex sample preparation steps, have hampered the implementation of these methods in the clinical settings. Ideally a diagnostic platform should be simple, accurate, rapid, and useable by non-technical staff for bedside diagnostics. Hence, rapid and reliable detection of bloodstream infections would gain a lot from improved and straightforward isolation of highly purified bacteria from whole blood. Towards this, we have developed a microdevice for rapid and selective lysis of blood cells while extracting intact bacteria for downstream analysis. The method exploits the fact that bacteria have rigid cell wall and can withstand harsher chemical treatment compared to blood cells.

EXPERIMENTAL

The experimental system consist of analyzing whole blood spiked with bacteria after flowing through a serpentine channel that enables rapid depletion blood cells via continuous chemical lysis while enriched bacteria are readily recovered for downstream analysis. The microfluidic device, fabricated using standard soft lithography methods [3,4], is shown in Fig1A. The lysis solution (chaotropic salts and/or detergents) branches into 2 streams, 1 on either side of the entry stream of whole blood to focus it into a narrow stream (Fig1B-C). Introducing a third solution (water) terminates the lysis. Bacteria samples (E.coli BL21 for gram negative and Micrococcus Luteus for gram positive) were analyzed by plating on agar plate overnight, while coulter counter was used to count blood cells.

RESULTS AND DISCUSSION

We have previously reported on microfluidic device for selectively lysing erythrocytes via exposure to di water while recovering white blood cells [3,4]. Here we apply similar strategy for the isolation of intact, viable and readily differentiable bacteria from whole blood. The microfluidic device (Fig 1) has two functional parts: the first part mixes whole blood with a

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lysis solution. Here selective blood cell lysis occurs. In the second part, a termination solution is added to immediately store the lysis process. The channel with herringbone structures to enhance mixing, enables controlled and rapid cell lysis (Fig.1B-C).

Initially, we tested a number of lysis solutions on a macroscale pilot study. Not surprisingly, we found it more difficult to lyse gram-positive bacteria (Micrococcus Luteus) than gram-negative (E.coli BL21). Moreover, for chaotropic salts, our study both at macroscale and microscale, suggests rapid lysis for both bacteria and blood cells at high chaotropic salt concentrations (>2M GTC) irrespective of exposure time. Even at reduced molarity (≤0.5 M), not all bacteria survive, as analysed by plating. In all cases, red blood cells were easily as compared to white blood cells. Similarly, Triton X100 was used to lyse all blood cells while bacteria survival depends on the amount of the detergent (Table 1). The highly variable in bacteria survival after 5 minutes treatment reveal TritonX100 to too harmful for bacteria plating.

Table 1: Bacterial survival after 5 minutes treatment with TritonX100.

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<thead>
<tr>
<th>TritonX100</th>
<th>Bacteria survival</th>
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<tbody>
<tr>
<td>0 %</td>
<td>2x10^3</td>
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<tr>
<td>0.1 %</td>
<td>25 %</td>
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<tr>
<td>0.5 %</td>
<td>5 %</td>
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<tr>
<td>1 %</td>
<td>20 %</td>
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<td>5 %</td>
<td>10 %</td>
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For sepsis diagnostics, the amount of bacteria present in the blood is very low – in the order of 10-100 cfu/ml. Hence it is imperative to recover all bacteria cells. Towards this, we have developed and optimized a novel two-step protocol that first exposes the whole blood to saponin followed by osmotic shock using DI water (Fig.3A-B). This treatment resulted in complete lysis of all blood cells, while 100% bacteria (both gram negative and gram positive readily recovered when exposed to 1-4% saponin, analysed by bacteria plating). Figure 2 shows plating results of E.coli bacteria after treatment of 1 and 2% saponin. Saponin forms pores in cell membrane bilayers, which leads to cell lysis. The DI water has dual function: it lyases the remaining white blood cells already damaged by saponin and terminates lysis for bacteria by diluting the saponin. Figure 3 shows a summary of the microfluidic-based protocol for selective blood cell lysis: the whole blood sample with spiked bacteria is first exposed to 2% saponin (10:1 ratio), followed by water treatment in (1:1 ratio). All bacteria are recovered irrespective of exposure time or incubation time after the water is added. We let the sample incubate for 30 minutes and plate to ensure the water does not harm the bacteria. Figure 3B shows Coulter count results of leukocytes for the different saponin contact times used, indicating > 99.5% cell lysis.
Figure 2. Bacteria recovery after incubation with the detergent saponin. E. coli bacteria plating results for different incubation times, 0-5 min, for saponin concentration of 1% (A-B) and 2% (C-D) indicate the bacteria are fully recovered and differentiate.

Figure 3. Microfluidic based selective blood cell lysis. (A) Bacteria plating following whole blood lysis. The plating results indicate bacteria are readily recovered and survive incubation time of 30 minutes after blood processing. (B) Coulter count results of leukocytes following selective cell lysis. 100 ml sample were analyzed immediately after being processed through the chip. The incubation time is for 2% saponing. PBS was used both for lysis and termination for the two control samples. Control one was analysed immediately, while control 2 was analysed after 5 minute of incubation after sample collection. More than 99.5% of the leukocytes are lysed. The scale is logarithmic.

CONCLUSION

In summary, we introduce a novel continuous-flow based microfluidic approach to isolate bacteria from whole blood. Here, we exploit the fact that bacteria have a rigid outer wall and can withstand harsher chemical lysis treatment compared to mammalian cells. Our approach lysis all blood cells, while ~ 100% of bacteria are readily recovered. Our method, as sample prep unit, can be of significant importance for the development of sensitive molecular based sepsis diagnostic tool.

REFERENCES


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