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A nanoparticle-based method for culture-free bacterial DNA enrichment from whole blood

Marwa M. Hassan, Andrea Ranzoni, Matthew A. Cooper*

Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland 4072, Australia

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ABSTRACT

Point-of-care (POC) diagnostics are one of the quick and sensitive detection approaches used in current clinical applications, but always face a performance tradeoff between time-to-result and assay sensitivity. One critical setting where these limitations are evident is the detection of sepsis, where 6–10 mL of whole blood may contain as little as one bacterial colony forming unit (cfu). The large sample volume, complex nature of the sample and low analyte concentration necessitates signal enhancement using culture-based or molecular amplification techniques. In the time-critical diagnosis of sepsis, waiting for up to 24 h to produce sufficient DNA for analysis is not possible. As a consequence, there is a need for integrated sample preparation methods that could enable shorter detection times, whilst maintaining high analytical performance. We report the development of a culture-free bacterial enrichment method to concentrate bacteria from whole blood in less than 3 h. The method relies on triple-enrichment steps to magnetically concentrate bacterial cells and their DNA with a 500-fold reduction in sample volume (from 10 to 0.02 mL). Using this sample preparation method, sensitive qPCR detection of the extracted *S. aureus* bacterial DNA was achieved with a detection limit of 5 ± 0.58 cfu/mL within a total elapsed time of 4 h; much faster than conventional culture-based approaches. The method could be fully automated for integration into clinical practice for point-of-care or molecular detection of bacterial DNA from whole blood.

1. Introduction

Point-of-care devices offer a low-cost detection approach that is carried out close to the patient (Dineva et al., 2007; Fournier et al., 2013; Gubala et al., 2012), particularly when detection time is a key (Dineva et al., 2007; Sharma et al., 2015). These devices are designed to simplify complicated laboratory-based methods in order to save handling time, whilst maintaining the target detection limit of more centralized large instrumentation (Gubala et al., 2012; Mark et al., 2010; Petryayeva and Algar, 2015; Turner, 2013). They require minimal manual handling of the sample, and may also be automated if required (Gubala et al., 2012; Mark et al., 2010; Petryayeva and Algar, 2015). Although there have been considerable advances in POC diagnostics for detection of infectious microorganisms, there are still some barriers for their clinical application and commercial development (Chin et al., 2012; Nayak et al., 2017). One challenge is the detection of target molecules in whole blood, especially those present in low abundance (Dineva et al., 2007; Gubala et al., 2012; Leggieri et al., 2010). The high concentration of cells in whole blood can block microfluidic channels, while dilution of the samples leads to subsequent dilution of target analytes, affecting the limit of detection. In some cases, shear stress in these microfluidic channels can cause red blood cells (RBCs) hemolysis, which can result in increasing the non-specific adsorption and interfere with detection (Cui et al., 2015; Toner and Irimia, 2005; Warkiani et al., 2015). Recently, a quick selective RBCs lysis method was used to facilitate detection of *Escherichia coli* (*E. coli*) TOP10 strain and *Micrococcus luteus* (10^6–10^4 cfu/mL) from 60 μL of 4-fold diluted blood samples (Zelenin et al., 2015). The time and the percentage of surfactants of the selective lysis treatment affected the percentage of lysed blood cells and bacteria (Zelenin et al., 2015), as both cell types are ruptured by the same methods. Thus, there is a need for efficient methods to selectively separate high levels blood cells from low levels of bacteria.

The scaling between the sample volume and the detection device core sensor presents another limitation for clinical application of POC devices. In POC devices, the sample volumes are often in small μL range (Dineva et al., 2007; Gubala et al., 2012; Leggieri et al., 2010), and the capacity to handle larger sample volumes is very limited (Gubala et al., 2012; Lee et al., 2007; Pitt et al., 2016). This is evident in the detection of bacterial sepsis, where quick and sensitive bacterial

* Corresponding author.
E-mail address: m.cooper@uq.edu.au (M.A. Cooper).

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detection is required from 6 to 10 mL of whole blood (Lee et al., 2007; Pitt et al., 2016). Additionally, the detection from larger blood sample volume (up to 20 mL) may be required in early sepsis cases to increase the sensitivity in such low bacterial counts (Lee et al., 2007). There have been a number of novel microfluidics approaches aimed to detect bacteria from large blood samples (reviewed in (Lui et al., 2009; Pitt et al., 2016; Tay et al., 2016)). One of these promising studies designed a microfluidic device to capture fluorescent labeled *E. coli* with on chip-antibodies and showed 70% capture efficiency at a flow rate of 2–10 μL/min with a detection limit of 50 cfu/mL (Wang et al., 2012). Surface acoustic waves have been used to separate a mixture of *E. coli* from blood mononuclear cells (Ai et al., 2013). Hou et al. designed a microfluidic device to separate bacteria from 15% diluted blood sample using the dean flow fractionation technique at a rate of ~ 20 min/mL. The device detected the mRNA of ciprofloxacin-resistant *E. coli* in 8 h with a detection limit of 10^5 cfu/mL (Hou et al., 2015). Another approach relied on different filtration methods to separate bacteria from larger volumes of whole blood. For example, a study filtered 80% of spiked bacteria from 10 mL 0.5% (v/v) blood at a rate of 200 μL/min (Mach and Di Carlo, 2010). Raub et al. separated 30% of the spiked bacteria from blood samples based on size exclusion microfluidic device with a maximum processing volume of 7.5 mL (Raub et al., 2015). Although all these methods showed progress towards clinical application for detection from large blood sample volume, but they still require further improvements to detect low levels of bacteria in blood that are clinically relevant.

Developed POC sepsis detection devices indicated remarkable lack of universal sample preparation methods in order to overcome the large sample volume limitation and improve the detection sensitivity. The developed POC approaches, described above, are not ideally suited to large sample volumes with maintaining the required selectivity to separate cellular debris from low bacterial counts (Cui et al., 2015; Gubala et al., 2012; Toner and Irimia, 2005; Warkiani et al., 2015; Yager et al., 2008). In contrast, magnetic nanoparticles (MPs) have been used to separate bacteria from blood (Lee et al., 2014; Strohmeier et al., 2015; Turner, 2013) owing to their ease of manipulation and the recent advancements in functionalization (Jamshaid et al., 2016). Superparamagnetic nanoparticles enable rapid analyte depletion from large sample volumes; however, the overwhelming abundance of erythrocytes can impair their capture efficiency (Veiseh et al., 2010). Molecular-based amplification techniques have also shown to be inhibited or lose their sensitivity by interferences from large blood sample volume (Bacconi et al., 2014; Banada et al., 2012; Hwang et al., 2011). In this study, we present Bac-ID, a culture-free bacterial enrichment method for concentration of bacteria and bacterial DNA from whole blood in 2.5 h. The method described herein is based on three enrichment steps to concentrate sensitive and resistant Gram-positive bacteria and further the bacterial DNA using magnetic nanoparticles. We showed efficient bacterial DNA extraction for all Gram-positive strains including capsulated strains. Also, we show diminished whole blood interferences and sensitive quantitative PCR detection of as low as 5 cfu/mL bacteria.

2. Experimental

2.1. Whole blood treatment

1 mL of sterile whole blood samples (obtained with agreement from Australian Red Cross Blood Service (ARCBS)) were diluted 1:1 with SSP+ solution (Macopharma) containing 2% dextran T500 (Pharmacosmos A/S) and 0.2% glucose (Sigma Aldrich) and incubated for 20 min at room temperature. After 20 min, the supernatant was aseptically transferred to a sterile Eppendorf tube.

2.2. Bacterial capture

The capture efficiency of each strain was assessed by spiking 10^2 cfu/mL bacteria in blood/platelets sample in a sterile falcon, 200 μL were sub-cultured on LB agar plate and considered as input. To capture bacterial strains, 2 μL of Van-NPs (50 mg/mL) (Hassan et al., 2017) were added to 1 mL of the spiked blood/platelets sample in a sterile Eppendorf tube. Then, all Eppendorf tubes were incubated for 1 h at 37 °C with rotation. The bacterial cells captured nanoparticles were washed 3 times with sterile phosphate buffered saline 0.1% Pluronic F127 (PBSP) from unbound bacteria, resuspended in 100 μL sterile PBSP and sub-cultured on LB agar plates. Cultured LB agar plates were incubated at 37 °C for 24 h and then the colonies were counted. Capturing efficiency was calculated based on the number of counted colonies of the output plates in comparison to the calculated input (cfu/mL).

2.3. Bacterial DNA enrichment

Magnetically captured bacterial cells were resuspended in 100 μL acetic acid (100 mM) (Merck) and incubated for 20 min at 65 °C, replicates were resuspended in sterile PBSP, sub-cultured on LB agar plates and incubated at 37 °C for 24 h as a control of bacterial count. After 20 min incubation, the Eppendorfs were allowed for magnetic separation and the supernatant was transferred to a new sterile Eppendorf and incubated for further 20 min at 65 °C. Then, 100 μL of 13 M guanidine HCl containing solution (Final concentration: 6.5 M Gu HCl (Sigma Aldrich), 10 mM Tris HCl pH 8.0 (Invitrogen), 1 mM EDTA pH 8.0 (Invitrogen), 5 mM Tris base pH 10.7 (Astral Scientific)) was added. Then, 6 μL of Si-MPs (MagPrep® Silica HS, Merck) were added and incubated for 15 min at 37 °C with rotation. DNA bound Si-MPs was washed with 80% isopropanol (Merck) magnetically. Then, 20 μL of 100 mM Tris HCl (pH 9.0) was added and allowed for DNA elution by incubating for 15 min at 65 °C. The 20 μL eluted DNA was transferred to a sterile Eppendorf and stored in − 20 °C.

3. Results and discussion

Bac-ID method (Fig. 1) is based on five steps: 1) aggregation of erythrocytes by treating whole blood with a dextran polymer solution, 2) bacterial capture using Gram-specific magnetic nanoparticles with washing to remove interfering cells, 3) magnetic-bacterial enrichment and elution, 4) bacterial lysis and DNA capture using silica nanoparticles, and 5) elution of concentrated, purified bacteria DNA.

3.1. Whole blood treatment

Whole blood treatment is the first step in Bac-ID method to aggregate RBCs. RBCs aggregation step was essential to decrease the percentage of hemolyzing and interfering cells. Whole blood samples were diluted 1:1 with a polymer aggregating solution. The time required for aggregation and the extent of reduction in blood cells was assessed by counting the blood cells at different time points using hemocytometer. The results showed 80% at 10 min and 98% at 20 min decrease in total RBCs and WBCs count/mL (Fig. 2A and Fig. S1). Remarkably, a reduction of interfering cells up to 99% cells/mL was demonstrated with slightly longer incubation times (30–40 min) (Fig. 2A and Fig. S1). The effect of RBCs aggregation on the bacterial count in whole blood samples was also studied. Whole blood samples were spiked with *S. aureus* and the bacterial count was assessed using culture at different time points of RBCs aggregation. RBCs aggregation caused loss of 26% and 43% of the spiked bacterial count at 10 and 20 min aggregation times (Fig. 2A), respectively. The bacterial count increased at the longer aggregation time of 30 and 40 min to 70% and 94%, respectively (Fig. 2A). This showed that aggregating blood cells affected the bacterial count and caused bacterial sedimentation.