Rapid separation of very low concentrations of bacteria from blood

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ABSTRACT

A rapid and accurate diagnosis of the species and antibiotic resistance of bacteria in septic blood is vital to increase survival rates of patients with bloodstream infections, particularly those with carbapenem-resistant enterobacteriaceae (CRE) infections. The extremely low levels in blood (1 to 100 CFU/ml) make rapid diagnosis difficult. In this study, very low concentrations of bacteria (6 to 200 CFU/ml) were separated from 7 ml of whole blood using rapid sedimentation in a spinning hollow disk that separated plasma from red and white cells, leaving most of the bacteria suspended in the plasma. Following less than a minute of spinning, the disk was slowed, the plasma was recovered, and the bacteria were isolated by vacuum filtration. The filters were grown on nutrient plates to determine the number of bacteria recovered from the blood. Experiments were done without red blood cell (RBC) lysis and with RBC lysis in the recovered plasma. While there was scatter in the data from blood with low bacterial concentrations, the mean average recovery was 69%. The gender of the blood donor made no statistical difference in bacterial recovery. These results show that this rapid technique recovers a significant amount of bacteria from blood containing clinically relevant low levels of bacteria, producing the bacteria in minutes. These bacteria could subsequently be identified by molecular techniques to quickly identify the infectious organism and its resistance profile, thus greatly reducing the time needed to correctly diagnose and treat a blood infection.

1. Introduction

Persistent over prescription and misuse of antibiotics over past decades has contributed to the evolution of multiple strains of bacteria resistant to even last-line antibiotics such as carbapenems and colistin (Sprenger and Fukuda, 2016; Papp-Wallace et al., 2011). This in turn has led to an increase in septic infections of resistant bacteria in patients (Martin, 2012; Levine et al., 1999). A rapid and accurate diagnosis of sepsis is vital to increase survival rates of those with bloodstream infections, particularly for those with carbapenem-resistant enterobacteriaceae (CRE) infections (Patel et al., 2008). Sepsis is a threat in the United States and worldwide, with total annual costs of $16.7 billion nationally and up to 19 million yearly cases across the globe (Angus et al., 2001; Adhikari et al., 2010). Despite the extensive research that has gone into preventing and treating blood infections, CRE-related sepsis is still associated with mortality rates as high as 50% (Patel et al., 2008). The extremely low levels of bacteria (1 to 100 cells ml^{-1}) found in a septic patient's blood increase the difficult challenges of rapid diagnosis (Reimer et al., 1997). Currently, hospitals initially respond by treating the patient with broad-spectrum antibiotics, which is often expensive, sometimes inadequate, and unfortunately contributes to increasing the prevalence of antibiotic resistance (Anonymous, 2013). The standard clinical procedure involves a blood culture followed by an assay, which typically takes at least 12–24 h to perform (Diekema and Pfaller, 2013). Since survival rates associated with CRE sepsis drop by as much as 9% each hour that the infection remains untreated (Garnacho-Montero et al., 2006), it is paramount that more rapid methods are developed so that the infectious organism and its resistance profile can be correctly identified as quickly as possible.

Researchers have developed various novel methods to identify bacteria in septic blood, but many of these processes may be difficult to quickly and effectively implement because of cost, efficiency, time, or limits of detection. Chemical and magnetic capture techniques have shown promising results with high bacterial concentrations ($\sim 10^3$ to 10^4 CFU/ml) and high removal efficiencies (Shen et al., 2016; Herrmann et al., 2015; Kang et al., 2014a). Drawbacks of these capture methods include that the binding agents must be specific to the bacteria, there must be a large number of beads to capture the low numbers of bacteria found in septic blood, and the process is often timeconsuming (Pitt et al., 2016). Few of these techniques have been demonstrated on blood with colony forming units (CFUs) in the range of 10–100/ml. In addition, the materials required in magnetic and chemical separation of bacteria from blood are often associated with a high cost (Pitt et al., 2016). Microfluidic devices have also received attention in the literature for successfully separating red blood cells (RBCs) from bacteria or other smaller particles using red cell

migration and particle focusing (Hou et al., 2016; Wu et al., 2009). These techniques typically employ small volumes of diluted blood, but could be scaled to higher blood processing rates using parallel flow devices (Pitt et al., 2016).

A high throughput of blood is necessary because the low concentrations of bacteria in a patient's blood require several milliliters of blood to be processed in order to collect enough bacteria for analysis. Additionally, the diagnostic process must produce rapid results because mortality increases rapidly with time. Finally, the technique must be reliable on the low numbers of bacteria found in a patient in order to have the detection sensitivity to be feasible in a clinical setting. Many of the published investigations involve experiments with thousands or millions of CFUs/ml – a high concentration that would rarely be found in a living person (Kreger et al., 1980; Leggieri et al., 2010; Yagupsky and Nolte, 1990). On the other hand, there are only a few investigations of bacterial separation from blood at clinically relevant concentrations of bacteria. For example, Kang et al. reported 77% recovery with 1 CFU/ml in blood using an Integrated Comprehensive Droplet Detection platform technology (Kang et al., 2014b). However, this was done on diluted blood. There are few methods shown to rapidly achieve this level of recovery on whole blood.

Our research group has developed a technique that successfully and inexpensively achieves rapid separation of bacteria from milliliter quantities of blood. However, those initial separation experiments (~10^6 CFU/ml) were not performed at clinically relevant (low) concentrations of bacteria in a patient's blood (Alizadeh et al., 2017). Therefore in this present report, very low concentrations of bacteria (originally 6 to 200 CFU/ml) were separated from blood using sedimentation in a spinning hollow disk. Due to the low number of bacteria, the plasma (containing the bacteria and some residual RBCs) was vacuum-filtered instead of using dilution and plating as our research group had done previously. Following vacuum filtration, the CFUs on the filters were counted to calculate the percent of bacteria collected from the original sample of human blood spiked with E. coli. This technique effectively separated bacteria from blood with concentrations of bacteria in blood as low as 6 CFU/ml in one minute of spinning.

2. Materials and methods

2.1. Solutions

Phosphate buffered saline (PBS, without calcium) was made from salts purchased from Sigma Aldrich (St. Louis, MO) and Fisher Scientific (Fair Lawn, NJ). PBS was sterilized by autoclaving. Sodium dodecyl sulfate (SDS, Acros Organics in Fair Lawn, NJ) was dissolved into distilled deionized water (DDH2O) at ~50 °C and cooled to make a 15% stock solution. Ethanol for disinfecting the spinning hollow disk was made by diluting 95% denatured ethyl alcohol (Fisher Chemical, Fair Lawn, NJ) with sterile water to a final concentration of 70% (vol/vol).

2.2. Preparing bacteria

Escherichia coli (E. coli, strain BL-21 Star DE3) was streaked from frozen culture onto a nutrient agar plate (DIFCO Sparks, MD) and incubated at 37 °C for 24 h. From this plate, a single colony was inoculated into 1 ml of nutrient broth (DIFCO Sparks, MD) in a sterile test tube. After shaking for 8 h at 200 rpm at 37 °C, this 1 ml bacteria suspension was transferred to 50 ml nutrient broth in a 250-ml sterile shaker flask. This flask was shaken for another 16 h at 200 rpm and 37 °C. The bacteria were washed twice by centrifugation (Horizon Model 642E, Fisher HealthCare, Fair Lawn, NJ, 1575 ×g for 10 min at room temperature) and resuspension in sterile PBS. The bacterial concentration was estimated by optical density at 600 nm using a spectrophotometer. The bacteria were diluted with sterile PBS to form a stock solution of the desired concentration.

2.3. Preparing blood for spinning

Following an IRB-approved protocol, and after informed consent of volunteers, blood was collected on the day of the experiment from healthy human donors into 10-ml EDTA anti-coagulant tubes (Vacutainer #366643, Becton Dickinson, Franklin Lakes, NJ). Immediately before experiments, the tubes were inverted several times by hand and 8.0 ml of blood was pipetted into another sterile test tube. A volume of 100 μl of the E. coli stock solution was added to the blood and mixed by lightly vortexing for 10 s.

2.4. Separation by sedimentation

To separate the plasma and bacteria from the RBCs and white blood cells (WBCs), a 12-cm-diameter spinning hollow disk was used (see Fig. 1). These acrylic disks were designed and printed in our lab using a rapid prototyping instrument (Object 30 Prime, Stratasys, Eden Prairie,
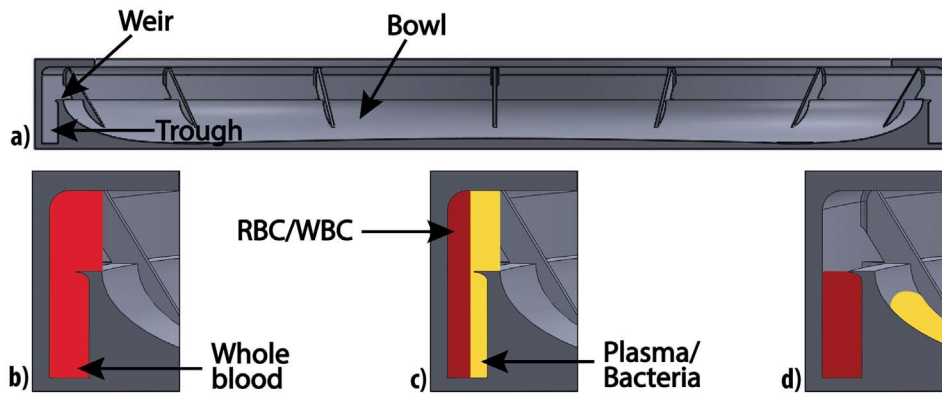
Fig. 1. Schematic of spinning blood in a hollow disk. a) Cross section of empty disk showing bowl, trough and weir. b) Upon spinning, blood is spun to the periphery of the disk. c) After 45 s at 3000 rpm, the RBCs and WBCs have sedimented quickly to form an outer layer, while most of the bacteria remains in the plasma layer. d) At conclusion of slow deceleration, the cells slide down into the trough while the plasma containing bacteria flows over the edge of the weir into the bowl where it is collected.
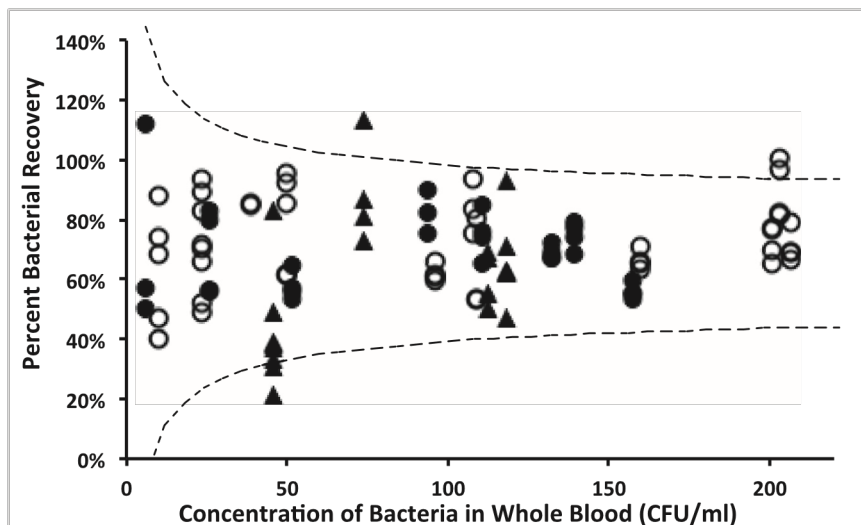

Fig. 2. The % recovery as a function of CFU/ml in blood for 98 experiments. Triangles (▲) represent data from female blood that was quantitated without lysing the RBCs. The circles represent data from female (●) and male (○) blood with the RBCs lysed before filtering. The dashed lines indicate an estimated 95% prediction interval as a function of bacterial

concentration.

MN, USA). The disk has a partial lid, a weir, and a hollow chamber on its periphery (see Fig. 1). During spinning, the blood flows into the chamber and forms a 2-mm thick film. Immediately the RBCs and WBCs start sedimenting toward the outer wall. When the disk slows down, the RBCs and WBCs slump into a trough behind the weir, and the plasma (containing bacteria) spills over the top of the weir into a collection bowl built into the mid-section of the disk (Alizadeh et al., 2017). The disk was repeatedly spun by programming an Arduino Uno (ATmega 328P) microcontroller. The output of the Arduino was directed to a L298N stepper motor driver (DROK Electronics), which drove a KP4M023 stepper motor (Japan Servo Co.). This stepper motor was directly attached to the output control dial of a power supply (KPS 620M, KEPCO INC, Sanford, New York) via adhesive tape. The power terminals of the power supply were connected to the OT-11400 CD spindle motor (Wagner Electronics Super Store, Ashfield, Australia) used to spin a platform to which the disk was attached. This setup allowed for programmable changes in the Arduino to repeatedly and precisely control the voltage output, and the corresponding rotational velocity of the disk.

Before spinning, the hollow disk was washed with water and disinfected with 70% ethanol spray, followed by drying with compressed air. Then 7 ml of the blood and bacteria mixture were pipetted into the hollow disk along with 1.5 ml sterile PBS. The disk was accelerated for 22 s to 3000 rpm and held at that speed for 45 s. The disk was decelerated slowly such that the separated plasma and red cells would not re-mix. When the disk came to rest, the plasma and bacteria drained into the bowl of the disk and were pipetted into a preweighed sterile test tube. The tube was weighed again to determine the weight of the plasma, which was converted to a volume of plasma assuming a density of 1.024 g/ml.

2.5. Quantitation by filtration

The CFUs in the recovered plasma were quantified using a filtration system placed in a sterile laminar flow hood. A 47-mm black filter with 0.45-μm pores (MSP000814, EMD Millipore, Billerica, Massachusetts) was placed on the porous metal filter connected to a Microfil Filtration System (1217N109, EMD Millipore, Billerica, Massachusetts). The membrane filter was sandwiched between the metal filter and a 100ml Microfil funnel (EMD Millipore, Billerica, Massachusetts) and wetted with sterile PBS. An aliquot of the bacterial suspension (either recovered plasma or stock suspension) was pipetted onto the filter, and the liquid was pulled through the filter via the vacuum. This was followed by a rinse of sterile PBS. Often the filtration was spread over 3 to 4 filters and funnels to avoid clogging the filter by residual RBCs. The filters were carefully removed and placed onto nutrient agar plates and the bacteria were allowed to grow for 24–48 h at 37 °C before counting colonies.

2.6. Filtration without lysis

2.6.1. Stock solution CFU/ml

To calculate the CFU concentration of the stock solution, 100 μl of the stock solution of bacteria was added to a test tube containing 8.0 ml of sterile PBS to prepare a bacterial concentration matching that of the whole blood that would be processed in parallel and counted similarly. This suspension in PBS was filtered and grown on plates as described above. The numbers of colony forming units (CFUs) from all filters were summed to determine the total number of bacteria in the 8-ml suspension, from which was calculated the concentration of the stock solution that was inoculated into the whole blood. This process was done in triplicate and the three values were averaged to estimate the initial concentration of bacteria in the corresponding whole blood samples that were spun on the hollow disk.

2.6.2. Counts in recovered plasma
Following spinning, the recovered plasma was serially diluted with PBS to various concentrations because in most experiments the undiluted recovered plasma had too many RBCs and platelets for the entire sample to be filtered. Samples of both undiluted plasma and the diluted plasma were vacuum filtered and grown as above. In examining the CFU counts, it appeared that in the undiluted plasma there was growth inhibition compared to counts from the diluted plasma, as there were much fewer CFUs/ml calculated in the undiluted plasma samples. Since hemoglobin in red cells is known to inhibit bacterial growth, we suspected that a number of RBCs were trapped with the bacteria on the filter surface, which might inhibit growth in the undiluted plasma (Liepke et al., 2003; Parish et al., 2001). As a result, the CFU counts from the undiluted spun plasma samples were excluded from the calculations, producing the data in Fig. 2. However, results from diluted plasma samples were included in the calculation of bacterial concentration.

To calculate the bacteria recovered from experiments using these diluted plasma samples, the number of CFUs on the filter was multiplied by the dilution factor and the ratio of the volume of recovered plasma to the volume of filtered plasma, thus estimating the number of CFUs in all the recovered plasma. This number of CFUs in the recovered plasma sample was divided by the total number of CFUs estimated to be in the whole blood, thus providing a calculation of the percent recovery from the blood sample.

2.7. Filtration with lysis

Because there was evidence (as described in the previous section) that residual red blood cells that were captured during filtration may have inhibited bacterial growth, most experiments were done by first lysing residual red blood cells in the recovered plasma before filtration so that the hemoglobin would flow through (and not remain on) the filter during colony growth. Sedimentation in the spinning disk reduced the concentration of RBCs in the recovered plasma by almost 2 orders of magnitude (Alizadeh et al., 2017), but many RBCs still remained. Lysing was done by adding DDH2O and hypotonic SDS solution to the plasma before filtering as described below.

2.7.1. Stock solution CFU/ml
To calculate the bacterial recovery from spun blood, we had to calculate as precisely as possible the number of bacteria added to the blood. To do this, a dilute stock solution of washed bacteria was prepared in PBS, and equal aliquots were added both to whole blood and to an equal volume of centrifuged clear plasma from the same donor. Thus any inhibition or promotion of growth due to plasma proteins would be consistent in these samples. To prepare the centrifuged plasma, two tubes containing 8 ml of blood each were spun in a centrifuge (Horizon Model 642E, Fisher HealthCare, Fair Lawn, NJ) at 3328 rpm for 20 min. Then 4.0 ml of clear plasma was pipetted off each tube and combined to place 8.0 ml in a sterile container. To this 8.0 ml of centrifuged plasma, 100 μl of the stock bacterial suspension was added to bring the bacterial concentration to match that of the whole blood used in sedimentation experiments. After a period of 5 min to an hour, this centrifuged and spiked plasma was first diluted 1:10 with DDH2O, and then SDS was added from a stock solution of 15% (w/v) to make the final concentration of 0.025% (w/v) SDS in the diluted plasma solution. The entire plasma sample was divided into aliquots, and each aliquot was vacuum filtered and the filters grown on plates as described previously. The numbers of bacterial colonies on each filter were summed to estimate the CFU/ml of the stock suspension of bacteria that was also inoculated into the whole blood used in spinning experiments.

2.7.2. Counts in recovered plasma

As described above, 8.0 ml of fresh human blood was spiked with 100 μl stock bacterial suspension and spun for 45 s at 3000 rpm on the hollow disk. Following the spinning, the recovered plasma was also diluted 1:10 with DDH2O in parallel with (at the same time as) the centrifuged plasma spiked with bacteria as described above. SDS was added from a stock solution of 15% (w/v) to make a concentration of 0.025% (w/v) SDS in the diluted plasma solution. The entire plasma sample was again divided into aliquots and each aliquot was vacuum filtered and grown on plates as described above. The numbers of colonies on each plate were summed to calculate the total number of CFU contained within the recovered plasma. To calculate the percent bacterial recovery, the total number of bacteria in the recovered plasma sample was divided by the total number of bacteria estimated to be in the volume of whole blood pipetted into the hollow disk.

2.8. Data analysis

Student t-tests were used to determine the difference between recovery in male and female plasma and to determine the difference between the results of the lysis and non-lysis experiments. The differences were not considered to be significant if p-values were greater than 0.05. A linear regression was applied to the data set to determine if there was any correlation between the percent recovery and the initial concentration of bacteria in the blood.

3. Results

We successfully separated low concentrations of bacteria from blood by spinning the blood quickly in a hollow disk and then removing the plasma, which still contained much of the bacteria and platelets, and some RBCs. We quantified the recovered bacteria by filtering the plasma and incubating the filters on nutrient agar plates. The initial number of bacteria in the blood sample was estimated from the volume of stock bacterial suspension added to blood, and its concentration. The % recovery was calculated by dividing the recovered number by the estimated number of bacteria in the blood placed in the hollow disk. The data are shown in Fig. 2.

One of the first questions to answer in this data set is whether there is any trend in bacterial recovery as the bacterial concentration increased. A linear regression was performed for each data set (nonlysis female, lysis female, lysis male). It was determined that none of the data sets had any statistically significant correlation with CFU/ml. Therefore the data within each set was averaged over the entire range of initial bacterial concentration (6–206 CFU/ml).

The average and 95% confidence interval for the non-lysis female and lysis female experiments were 60 ± 10% (n = 22) and 69 ± 5% (n = 30), respectively. As these values are not significantly different from each other (p = 0.35, two-sided t-test), they were combined into a female group (65 ± 5%, n = 52) and compared with the recovery values from experiments with male blood (73 ± 4%, n = 46). These values are not significantly different from each other (p = 0.29, twosided), so they were combined for an overall mean and 95% confidence interval of 69 ± 3% (n = 98).

4. Discussion

The recovery of 69% of bacteria from blood with very low CFU in only 1 min of spinning on a simple device is remarkable, particularly considering that E. coli bacteria and RBCs cannot be separated by isopycnic centrifugation due to overlapping ranges of densities (Pitt et al., 2016). Conventional centrifugation would not separate bacteria from blood. However, dynamic sedimentation provides transient separation, which is influenced more by particle size than by density. A short but rapid spin provides sufficient separation such that the bacteria left in the plasma can be subsequently isolated by filtration, even though platelets and some RBCs are present.

4.1. Lysis vs non-lysis experiments

Some experiments were performed with lysis of the red blood cells by DDH2O and SDS because studies have shown that hemoglobin may inhibit bacterial growth (Liepke et al., 2003; Parish et al., 2001). Lysing the RBCs was done to minimize any hemoglobin trapped on the filter with the bacteria. Optical microscopy showed that the 1:10 dilution with DDH2O used in these experiments swelled the RBCs and lysed some of them. However, adding 0.025% SDS lysed nearly 100% of the RBCs as evidenced by the absence of any red color on the filters. Apparently this amount of SDS did not affect the growth of the bacteria, perhaps because SDS was also washed through the filter. This lysis of the RBCs allowed hemoglobin to be released and washed through the filter so that it was not trapped on the filter and present in high quantities when bacteria started to grow. In any event, in our experiments, we found there was no difference in the calculated bacterial recovery of experiments done with and without RBC lysis. This is attributed to: 1) we did not use data from filtrations of undiluted recovered plasma as preliminary experiments showed some signs of growth inhibition; 2) the concentration of RBCs in diluted recovered plasma was insufficient for hemoglobin to measurably inhibit bacterial growth on the filters.

4.2. Scatter

Recovering bacteria from blood at concentrations as low as 6 CFU/ ml presents a great challenge, not only in physical recovery, but also in accurate counting of such low numbers. Not unexpectedly, results from experiments using low concentrations of bacteria show higher scatter patterns than previous experiments using higher bacterial concentrations (Alizadeh et al., 2017). This can be explained by the statistical rule that there is more variability in single sampling estimates of a concentration that has low counts.

Whether a bacterium is retained in the recovered plasma or trapped in the cell pack remaining in the disk is a stochastic process. When the bacteria are at such low concentrations (6 CFU/ml) and numbers (42 CFU in 7 ml of blood), the stochastic variation of just a few bacteria counted from experiment to experiment has a large impact on the % recovery. The data in Fig. 2 show an occasional bacterial recovery rate over 100% for a single experimental calculation. In such cases, we are quite confident in the number of bacteria recovered, as they were directly measured. As explained, in the lysis experiments all of the recovered plasma was filtered, giving high credibility for the total number of CFUs in the sample. However, the initial bacteria placed in the hollow disk is an estimate based on the calculation of the concentration of bacteria in the stock suspension, which might be biased low due to normal scatter in counts within the 100 μl volume of stock suspension. A low estimate of the stock suspension concentration would produce a high estimate of bacterial recovery, and could push that estimate over 100% in some cases. However, if such scatter in estimating stock solution concentrations is normally distributed, there would be other experiments with high bias in the estimate of the stock suspension, producing an underestimation of the true recovery. Therefore we did 98 experiments to get a good estimate of recovery. As mentioned there is stochastic scatter in the true recovery of 42 (7 ml at 6 CFU/ml) bacteria scattered randomly throughout the disk during the spin. The probability of a single bacterium being recovered in the plasma or trapped in the red cells would depend on the initial radial placement of bacteria on the disk and its interaction with neighboring cells during sedimentation; and with only 42 bacteria this recovery would not be consistent in each experiment. Needless to say, the scatter is expected to be large at low CFU/ml, and to decrease as the concentration increases, which is consistent with the data in Fig. 2. The dashed lines in Fig. 2 represent prediction bounds that enclose 95% of the data in this set, using a model in which the variance in the data is the sum of variance due to counting low numbers, and a constant variance due to execution of experimental procedures.

4.3. Recovery

It is curious that the same type of experiments, employing the same spinning disk, processing human blood spiked with much higher concentrations of E. coli ($\sim10^6$ CFU/ml) gave a somewhat lower recovery of about 36% in our previous experiments (Alizadeh et al., 2017). Our initial hypothesis was that the recovery rates should be the same, independent of the concentration of bacteria. Our surprise at finding a higher recovery rate at these lower bacterial concentrations is shown by the 98 repetitions of this experiment. At present, we have no good explanation of why blood samples spiked with extremely low counts show better recovery of bacteria in the same mechanical system. One of the few differences between the experiments discussed in this paper and the experiments previously performed by us at higher CFUs is that the previous results were determined by serial dilutions and plate counting of the samples, and the results of these present experiments were measured by direct filtration of the plasma and counting of colonies on the filter. The use of filtration was necessary because the entire recovered volume ($\sim$3.7 ml) was processed, and conventional dilution and plating of this volume of sample with low concentrations of bacteria would produce a cumbersome experiment with such low numbers of bacteria per plate that the observed numbers would be statistically questionable. Both of these methods (filtration and serial dilution and plating) are accurate ways of determining recovery and in theory should not contribute to the difference in results that is seen here. In addition, our prior experiments used bacteria in whole blood to estimate the concentration of stock suspensions, while these experiments used bacteria in centrifuged plasma because whole blood could not be filtered. The extra platelets and RBCs present in our previous experiments in theory should not make a difference in estimating suspension concentration, as the bacteria were in whole blood (instead of plasma) only a short time (on the order of tens of minutes) and should not have grown significantly more than when suspended in plasma. However, if in the previous experiments, bacterial growth in whole blood occurred faster than growth in plasma, the resulting estimate of % recovery (for previous experiments) would be biased toward a lower value than calculated in these present experiments. A lower value of recovery was indeed observed in the previous experiments than in the present experiments.

Careful scrutiny of our own procedure reported herein reveals another small inconsistency. In the lysis experiments, the stock suspension concentration is calculated using bacteria suspended in centrifuged plasma that has no platelets. However, the recovered plasma contains many platelets and some RBCs (Alizadeh et al., 2017). The presence of platelets may have produced an effect during filtering the recovered plasma that was not present when filtering bacteria in clean plasma. However, we would expect that platelets might decrease the observed colony counts by binding to two bacteria and making only 1 CFU instead of 2 colonies. Likewise, WBCs in the blood (but not the plasma calibration experiments) may have prevented some bacteria from growing. But again, these errors would have produced a bias toward a lower estimate of % recovery, instead of producing a higher estimate. Similarly, the residual RBCs in the spun plasma might have inhibited growth and decreased the observed number of CFU; but again, this would have resulted in a lower estimate of % recovery.

To summarize, from our analysis of the imperfections in the present experiments and the differences between these experiments and prior experiments done at higher concentrations, we have been unable to determine a reason for the unexpectedly higher recovery in these experiments using direct filtration instead of serial dilution and plating. In fact, the analysis suggests that the % recovery in these low-CFU experiments provides

a better estimate of true recovery than the prior experiments done at much higher CFU/ml. Furthermore, these low-CFU experiments were done at clinically relevant concentrations of bacteria in blood.

4.4. Implications

We have shown that our sedimentation method using a spinning hollow disk is successful at separating about 69% of low concentrations of bacteria in about a minute. This technique can be used at clinically relevant levels of bacteria. Using our RBC lysis technique, the bacteria can be quickly recovered by filtration and is immediately available for analysis. For example, the bacteria could be quickly collected from the filter, lysed to release their genomic and plasmid DNA, which could then be subjected to multiplex PCR with primers designed to identify species and antibiotic resistance genes.

Because the % recovery was not correlated with the concentrations of bacteria in whole blood, one can assume that further experimental development (e.g. disk design, spin time and spin speed) can be done with higher bacterial concentrations using the filtration method presented herein, with the assurance that the data derived from higher-concentration experiments will be applicable to the low clinical levels of bacteria in blood. This can save time and resources in future experiments.

We posit that our method of using a spinning hollow disk is the first step in the development of an assay for rapid isolation of bacteria from whole blood, and that subsequent molecular techniques can easily be employed for identification of bacterial species and their resistance profile.

Funding declaration

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