Improving and Modeling Bacteria Recovery in Hollow Disk System

Clifton Anderson

Brigham Young University

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Improving and Modeling Bacteria Recovery in Hollow Disk System

Clifton Anderson

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

William G. Pitt, Chair
David O. Lignell
Douglas R. Tree

Department of Chemical Engineering
Brigham Young University

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ABSTRACT

Improving and Modeling Bacteria Recovery in Hollow Disk System

Clifton Anderson
Department of Chemical Engineering, BYU
Master of Science

Identifying antibiotic resistance in blood infections requires separating bacteria from whole blood. A hollow spinning disk rapidly removes suspended red blood cells by leveraging hydrodynamic differences between bacteria and whole blood components in a centrifugal field. Once the red cells are removed, the supernatant plasma which contains bacteria is collected for downstream antibiotic testing.

This work improves upon previous work by modifying the disk design to maximize fractional plasma recovery and minimize fractional red cell recovery. V-shaped channels induce plasma flow and increase fractional plasma recovery. Additionally, diluting a blood sample spiked with bacteria prior to spinning it increased the fractional bacteria recovery. A numerical model for red cell sedimentation shows that red cells are removed from solution more rapidly as the blood is diluted. Diluting blood is beneficial but may create too much biological waste. The benefits of diluting are formulated as an optimization problem subject to the end user’s needs.

Keywords: sepsis, bacteremia, antibiotics, blood, sedimentation, modeling conservation laws
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1 INTRODUCTION

Blood-stream infections (BSI) develop quickly and have high mortality rates. Severe infections can be difficult to diagnose [1], but their likelihood of developing into septic shock increases by 7.6% per hour when left untreated [2]. Septic shock causes death in 50% of patients, even after treatment in an intensive care unit [2, 3]. Most forms of septic shock can be treated with antibiotics, but some bacterial infections are resistant, leading doctors to empirically treat initially, sometimes with the wrong antibiotic. Immediate antibiotic treatment is frequently recommended [2, 4], but an emerging need of better antimicrobial stewardship is motivating research for specific, yet rapid identification of correct treatments [5-7]. Additionally, although BSI result from some pre-existing infections, the infection source and microbe identity are unknown in 30-70% of cases [8-11]. Current methods to detect bacteria require 12-36 hours [12, 13], in which blood samples are incubated until the bacterial population becomes measurable. This process takes so long because BSI can contain as few as 1-10 bacteria (a.k.a. colony forming units, or cfu) per mL of blood. In addition, some blood components mask the presence of bacteria and hinder microbiological tests for antibiotic resistance. Separating bacteria from the blood removes that hindrance.

Blood cells make up 30-55% of blood volume and greatly outnumber bacteria. The formed elements of blood are ~99 vol% red blood cells, 1% platelets, and 0.2% white blood cells by number. The volume fraction of red blood cells is called hematocrit. To further complicate the
problem of separation, bacterial and red blood cell specific gravities tend to overlap. Fortunately, their hydrodynamic properties do not. Red blood cells (RBCs) sediment about 30 times faster than bacteria because they are larger than most bacteria (5-8 µm diameter vs 1-4 µm for RBCs and bacteria, respectively). Most methods reported in the literature leverage hydrodynamic differences to separate blood cells and bacteria. For example, Dr. Pitt’s group has developed a centrifugal disk that uses 7 mL of blood and quickly sediments the RBCs out of suspension, leaving bacteria in the plasma [14]. This process takes about 5 minutes, and the bacteria-laden plasma has fewer red cells than the original whole blood sample.

Previous work on this separation process focused on disk modifications to recover more plasma and fewer red cells, as well as exploring the effect of spin time and disk speed (angular velocity) on bacterial recovery. It succeeded in recovering an average 70% of the plasma, 5-20% of the red cells, and 60% of the bacteria during a total spin time of ~3 minutes.

This work pursues similar aims. Its primary objectives are to maximize recovered bacteria and minimize red cells in the recovered plasma. It focuses on (1) iterative improvements for recovering more plasma and fewer red cells and briefly explores the effect on plasma recovery of polymer coatings on the disk. Another main objective is (2) to explore how diluting the blood prior to spinning affects optimal spin time and bacteria recovery. Finally, it aims to (3) develop a numerical model of simultaneous sedimentation of particles in blood to explore and optimize process design.
2 LITERATURE REVIEW

This chapter begins by describing how body forces and solids concentration affect the speed of blood cells and bacteria in a suspension. It then sets up the mathematical framework for describing sedimentation and finishes by giving a brief overview of integration methods used in this thesis. It does not focus on contemporary technology for identifying antibiotic resistance. For that, the reader is referred to a recent article by Pitt et al. [15].

In this thesis, the term “suspension” is defined as a mixture of fluid and particles in which the particles are not in continuous contact with each other or with the wall. In this thesis, it most commonly refers to a layer of cells (potentially of different type) surrounded by blood plasma. In contrast, the term “sediment” refers to the region in the spatial domain comprised of cells that are either touching the wall or that are in continuous contact with cells that are touching the wall. “Sedimentation” refers to the movement of cells in the direction of body force applied by a gravitational or a centrifugal field. Finally, the “clear fluid” region has no suspended cells.

2.1 Stokes’ law

The steady state velocity of particles in a centrifugal field can be predicted at Re<<1 using Stokes’ law (Equation 2-1). This law gives the drag force on a rigid sphere in creeping flow at infinite dilution (i.e. no interparticle interactions) as a function of fluid viscosity (\(\mu\)), particle diameter (\(D\)), and particle velocity (\(u\)) relative to the suspending fluid:

\[ F_d = 6\pi\mu Du \]
\[ F_{\text{drag}} = 3\pi \mu u D. \]  

When balanced against the centrifugal body force, Stokes’ law predicts the particle’s terminal velocity, or “Stokes velocity”:

\[ F_{\text{body}} = \frac{\pi}{6} D^3 \omega^2 r (\rho_{\text{particle}} - \rho_{\text{fluid}}), \]  

\[ u_\infty = \frac{\omega^2 r D^2 (\rho_p - \rho_f)}{18 \mu} K, \]

where \( u_\infty \) is the particle velocity at infinite dilution, \( \omega(t) \) is the angular velocity, \( \rho_p \) and \( \rho_f \) are the particle and fluid density, respectively, \( r \) is the distance between the particle and the axis of rotation, and \( K \) is an empirical correction factor used when conditions for Stokes flow are not met. Physical properties of red blood cells and bacteria are given in Table 2 of Appendix A.

Many equations have been developed that modify the Stokes velocity to account for nonideality in sedimentation. A scalar shape factor, \( K \), can be used to modify the Stokes velocity for non-rigid, non-spherical shapes [14]. These shape factors describe dilute sedimentation satisfactorily [16], but when a suspension concentration rises above 4 volume percent, the Stokes velocity overpredicts true sedimentation velocity. Further corrections have been made to account for higher particle concentration and non-rigid bodies.

2.2 Hindered settling corrections

Hindered settling corrections (HSC) consider how particle interactions in concentrated suspensions inhibit particle movement. These corrections reduce the sedimentation velocity to some fraction of the calculated Stokes velocity. Correlations exist for a broad range of particle sizes and flow regimes, but this present description will be based on HSC applicable in creeping flow. These correlations all decrease the settling velocity to zero in the limit of high particle
density: \( \lim_{\phi \to \phi_{\text{max}}} \frac{u}{u_\infty} = 0 \), where \( \phi \) is the volumetric particle concentration. Many authors have investigated the hydrodynamics of dilute and concentrated suspensions of rigid spheres [17-24]. When modeling meso to macro scale sedimentation specifically, the most common HSC range from empirical to semi-empirical for practical conditions [25-33].

The most common empirical HSC in sedimentation literature is likely the Richardson-Zaki (RZ) correlation. It is a simple power law based on void volume, or the volume of liquid between particles:

\[
\frac{u}{u_\infty} = (1 - \phi)^n.
\]

Here, \( \phi \) is the volume fraction of the suspended particles, and \( n \) is a constant that varies with the Reynolds number [34]. For RBC centrifugation, this value varies between 2.5 and 3.5 [25, 35]. This correlation is widely used for its simplicity and applicability, but it has a few problems. First, it predicts non-physical velocities at concentrations higher than the maximum particle packing. For instance, the maximum packing factor of random rigid spheres is 0.65, but the RZ correlation predicts positive velocities until a particle volume fraction of 1, which is impossible for solid particles. As a result, many people use a piecewise form where the velocity is truncated to 0 at \( \phi > \phi_{\text{max}} \). Physically, this says that particles move at constant speed until they collide with the bed of stationary particles at the wall. In this thesis, sediment is defined as the layer of cells that are in constant contact with the wall or with other cells that are touching the wall. For systems involving flexible particles, the sediment compresses with time and the interface may not be sharp between suspension and sediment. For concentrations well below the sediment concentration, however, the RZ correlation predicts sedimentation velocities accurately and is widely used.
Corrections that are valid over the entire domain of concentrations have also been developed. These tend to be more theoretical, though still quite empirical. For example, the Michaels correlation is an amendment to the RZ correlation that restricts the domain to $\phi \in [0, \phi_{\text{max}}]$ [31]. It is as follows:

$$\frac{u}{u_{\infty}} = \left(1 - \frac{\phi}{\phi_{\text{max}}}\right)^{n_{\text{def}}\phi_{\text{max}}},$$

where $n_{\text{def}}$ is a parameter describing particle deformability. This equation predicts a zero velocity at maximum packing. Whereas the (clipped) RZ correlation physically implies that particles collide with a sediment and stop instantaneously to form a clear suspension-sediment interface, the Michaels correlation slowly decreases the particle velocity to zero and does not distinguish a cutoff $\phi$ between suspension and sediment. This is valuable because red blood cells are extremely flexible: reported maximum packing factors of RBCs range from 0.8 [36] to 0.97 [37] depending on the strength and duration of the centrifugal field. One group emphasized the slow compaction of the red cell sediment by combining the RZ and Michaels correlations in the following way [36]:

$$\frac{u}{u_{\infty}} = (1 - \phi)^2 \left(1 - \frac{\phi}{\phi_{\text{max}}}\right)^{n_{\text{def}}\phi_{\text{max}}}. $$

Here, the Michaels correlation is used to simulate the viscosity of the red cell sediment. Lerche et al. showed that this form accurately modeled RBC sedimentation and compaction for a broad range of hematocrits using $n_{\text{def}} = 2.71$. This model represents RBC sedimentation profiles well, especially at high hematocrit.

HSC also exist for particle mixtures. For example, the Masliyah-Lockett-Bassoon (MLB) [38] correlation is a generalized form of the RZ correlation. Another HSC describes the
“apparent porosity,” or average interparticle spacing as a concentration-weighted sum of particle diameters [35, 39].

2.3 Converting from slip velocity to absolute velocity

Stokes’ law and many HSC describe the slip velocity, or velocity of the particle relative to the fluid. However, a stationary reference frame models the particle velocity with respect to the disk instead of to the fluid. A volume balance on aggregate particle movement converts from slip velocity \( u \) to absolute velocity \( v \) [29]:

\[
v_i = u_i - \sum_{j=1}^{N} u_j \phi_j.
\]

for the \( i^{th} \) species (including plasma if desired) summed over all \( N \) species. The result does not change when plasma is considered because the slip velocity of plasma relative to itself is zero. The term in the summation is the volume average suspension velocity and represents backward fluid flow. Equation 2-7 allows us to calculate the plasma velocity. For a single particle type, this equation simplifies to:

\[
v = u(1 - \phi).
\]

Hence, the absolute velocity is always smaller than the slip velocity. When reading about an unfamiliar HSC, it is important to note whether it modifies the slip or the absolute velocity. Failure to do so can bias velocity estimates by a factor of \( (1 - \phi)^{\pm1} \).
2.4 Equation formulation

2.4.1 Conservation law formulation

The theory for sedimentation processes was pioneered by Kynch in 1954, who described the convective flux of particles along an accelerating field as a function of local concentration. In conservation form and Cartesian coordinates, the hyperbolic system of PDEs describing such particle sedimentation can be written as follows:

\[
\frac{\partial \Phi}{\partial t} + \nabla \cdot F(\Phi) = 0,
\]

where \( \Phi \) is a vector of \( J \) volume fractions for each possible cell type (e.g. red cells, white cells, platelets, bacteria). The vector function \( F(\Phi) \) is referred to in some literature as the “batch Kynch flux function” [28] and is the volumetric particle flux. It is the product of species volume fraction and absolute velocity (i.e. \( F_j(\Phi) = \Phi_j v_j(\Phi) \)). For multicomponent systems, the dependence of \( F(\Phi) \) on the entire vector of volume fractions \( \Phi \) makes this a coupled system of equations. This is a continuum approach that considers the cells as incompressible liquid phases and does not explicitly model the plasma. Thus, a system with one particle type suspended in fluid is called a 2-component system, but it is a scalar equation.

This model does not account for any particle diffusion [40], but some models have used a diffusive term to describe sediment compression [28, 41, 42]. Equation 2-6 given by Lerche does not consider the sediment specially. One benefit of casting the problem as the continuity equation is the wide base of knowledge for integrating conservation laws. Integration schemes can have very rigid constraints, however. Tory modeled multicomponent hindered settling using a stochastic particle-based approach that provided some extra modeling flexibility [43].
2.4.2 Characteristic structure and the sedimentation flux curve

Applying the chain rule to the 1D scalar (2 component) form of Equation 2-9 converts it into characteristic form:

\[ \frac{\partial \phi}{\partial t} + \frac{\partial}{\partial \phi} \frac{\partial \phi}{\partial x} = 0. \] 2-10

Here, \( f \) is the scalar form of the flux function \( F \). Characteristic form represents the continuity equation in a different perspective. Whereas the conservation form is reminiscent of material balances and the familiar divergence theorem, the characteristic form represents the continuity equation more like a wave. In characteristic form, the continuity equation becomes a wave of height \( \phi(x, t) \) that moves in the \( x-t \) plane at speed \( \partial f / \partial \phi \), or \( f'(\phi) \). Values of \( \phi \) remain constant along curves referred to as characteristics that have slope \( dx/dt = f'(\phi) \). If the flux function has no spatial dependence, then characteristics are straight lines. Characteristics are the path that the wave \( \phi(x, t) \) takes in the \( x-t \) plane. It is important to note that the speeds of characteristics are conceptually different than the physical speed of the red cells. Characteristics are a mathematical construct that represent the direction and speed of information propagation. They span the entire spatial and temporal domain until they either exit the domain or intersect with another characteristic.

When characteristics spread away from each other, a rarefaction wave forms through which \( \phi(x, t) \) varies smoothly. On the other hand, when characteristics intersect, a discontinuity in \( \phi \) forms with states \( \phi_L \) and \( \phi_R \) associated with the characteristics to the left and right of the discontinuity. This is known as a shock. The shock speed \( s \) is obtained by the Rankine-Hugoniot jump condition \( s = \frac{f(\phi_R) - f(\phi_L)}{\phi_R - \phi_L} \), which can be thought of as a material balance. With a given flux curve, however, the Rankine-Hugoniot condition can predict shock speeds that are not physically
valid. To prevent this, shock speeds are subjected to a so-called “entropy condition”. One such condition is the Oleinik entropy condition \(\frac{f(\phi) - f(\phi_L)}{\phi - \phi_L} \leq s \leq \frac{f(\phi) - f(\phi_R)}{\phi - \phi_R}\), where \(\phi \in [\phi_L, \phi_R]\).

What this says is that the shock speed is bounded by the speed of the characteristics that collided.

For non-convex flux functions, it is sometimes impossible to connect two states by a shock without generating unphysical solutions because the line connecting two states must not cross the flux curve. In this case, the unphysical shock is mathematically restated as a combination of shock and a rarefaction, which are both physically valid, connected by an intermediate state \(\phi_{rare}\). Finding the states across this new combined shock-rarefaction involves constructing a convex hull (see Figure 1) (a.k.a. the “stretched string analogy” or “rubber band method”) around the flux curve. The tangency point in the convex hull \(\phi_{rare}\) is the state on the rarefaction side of the shock in the new shock-rarefaction combination (see Section 16.1.2 of [44]).

Figure 1 shows the red cell flux curve and the characteristic structure of Equation 2-10 with initial hematocrit \(\phi_0\). The flux curve is not convex due to the high-order polynomial exponents in the HSC. Physically, the red cell flux is the volumetric rate of red cells crossing a plane perpendicular to the cells’ velocity. It is the product of red cell volume fraction and velocity. In the limit of infinite dilution, all the cells move at their maximum speed. However, red cell flux is low because there is low red cell volume. As the suspension becomes more concentrated, the cells slow down due to hindered settling, but their total volume increases, so the red cell flux reaches a maximum near \(\phi_{flux max} = 0.17\) and decreases to 0 at \(\phi_{max} = 0.97\) as the velocity approaches 0. The characteristic speed begins at 1 for low hematocrit, decreases past...
0 at \( \phi_{\text{flux max}} \) to a minimum at the flux inflection point \( \phi_{\text{infl}} = 0.348 \) and slowly raises back up to 0 as \( \phi \) approaches \( \phi_{\text{max}} \).

Figure 1 Dimensionless red cell flux curve and characteristic speed versus hematocrit showing shock and rarefaction curves. Blue solid line shows red cell flux normalized by red cell Stokes velocity using Lerche’s hindered settling correction. Green dashed line shows the derivative of the red cell flux showing the speed of characteristics versus wave height (i.e. hematocrit). Black line with points shows the convex hull that connects each state by shocks (straight lines) and rarefaction (curved portion).

An interesting result of the characteristic form is that shocks represent interfaces between different regions of the suspension. Consider the initial profile \( \phi(x, t) = \phi_0 \) with boundary conditions \( \phi(0, t) = 0 \) representing clear plasma and \( \phi(x = L, t) = \phi_{\text{max}} \) representing compacted cells at the disk wall. At \( x = 0 \), there are no red cells; the characteristic speed is +1, and the value \( \phi = 0 \) enters the domain from the left for all \( t \). In the middle of the domain, the initial profile \( \phi(x, t) = \phi_0 \) advects along characteristics with the speed \( f'(\phi_0) \), but it is overtaken by the faster-moving characteristics coming from the boundary at \( x = 0 \). This forms a
shock with the states $\phi_L = 0$ and $\phi_R = \phi_0$ which moves at the speed $s = \frac{f(\phi_0) - f(0)}{\phi_0 - 0} = \frac{f(\phi_0)}{\phi_0} = v(\phi_0)$. This shock is the suspension-clear fluid (SC) interface.

A more complex interaction between characteristics occurs on the right side of the domain ($x = L$) where the right boundary $\phi_{\text{max}}$ is a stationary wave ($\partial f / \partial \phi = 0$). The initial discontinuity between $\phi_0$ and $\phi_{\text{max}}$ cannot generate a shock without violating an entropy condition, and a combined shock-rarefaction occurs, as previously described. Between the states $\phi_0$ and $\phi_{\text{rare}}$, a shock forms that represents the interface between suspended red blood cells and the beginning of a red cell sediment. This is the suspension-sediment (SS) interface, and it moves with speed $s = \frac{f(\phi_{\text{rare}}) - f(\phi_0)}{\phi_{\text{rare}} - \phi_0}$. On the right side of the interface is the sediment, in which $\phi$ varies smoothly from $\phi_{\text{rare}}$ to $\phi_{\text{max}}$. The value of characteristics is that the SS and SC interfaces can be tracked simply by finding shock speeds.

2.5 Integration

2.5.1 Method of characteristics

The method of characteristics is an analytical method for solving hyperbolic equations by tracing the characteristics of a hyperbolic equation to find a final solution. For simple scalar conservation laws, the solution may be a simple piecewise function. In fact, some authors have solved sedimentation problems using this method by explicitly tracking interface positions [45, 46]. Using the method of characteristics for systems of nonlinear equations, however, involves decoupling the system by diagonalizing the Jacobian matrix $\partial F / \partial \Phi$ and expressing $\Phi(x, t)$ as a linear combination of the Jacobian’s eigenvectors.
Notably, Sartory solved the sedimentation problem for a 3-component system of granulocytes (small white blood cells) and red blood cells [47]. His method was mostly analytical, and Figure 2 shows an example of his solution. The left axis shows dimensionless height with gravity pointing downward to the tube bottom. The original suspension is a mix of red blood cells at $\Phi_0^R = 0.25$ and white blood cells at $\Phi_0^W = 0.001$. As time progresses, a sediment layer consisting of red cells and granulocytes extends up from the tube bottom. Granulocyte concentration in this layer decreased because his equations accounted for them being less dense than red cells [30]. This differs from our system because bacteria and red cell densities overlap. His solution shows two distinct suspended layers: (1) the original mixture and (2) a pure granulocyte suspension whose concentration exceeds the starting concentration. Each layer travels at a constant rate until it reaches the sediment layer. Sartory measured the number of recoverable granulocytes as the thickness of the pure granulocyte layer multiplied by its concentration.

![Figure 2 Example of sedimentation profile for red blood cells ($C^R_0 = 0.25$) and white blood cells ($C^W_0=0.0011$). Interfaces: –-; rarefaction in the sediment: –--. Reproduced with permission from [47].](image)
Sartory’s result is very interesting for our process for several reasons. Although other authors have shown distinct suspended layers [48-50], Sartory’s solution highlights that concentration of the slower particle in its pure suspension was enriched relative to its starting concentration. That is interesting because it means that bacteria may be recoverable at a higher concentration than their initial concentration. We are aware of no other work that predicts a relationship between this enrichment of the second component in the supernatant fluid as a function of the the volume fraction of the first component.

2.5.2 Numerical integration

Numerical integration schemes for sedimentation need to account for discontinuities caused by interfaces and for non-convexity. Solutions to a $k^{th}$ order PDE that are $k$-times differentiable and satisfy the PDE are known as “strong” solutions. Traditional finite difference schemes derived from Taylor series assume strong solutions and can fail for problems with inherent discontinuities. Instead, dealing with an integrated form of the PDE removes the need for continuous derivatives. Solutions to the integrated PDE are called “weak” solutions because they only satisfy one form of the PDE. Weak solutions arise frequently in physical systems. Not all weak solutions are unique, however, which necessitates extra care via the entropy condition briefly discussed in Section 2.4.2.

Integrating the conservation law Equation 2-9 serves as the basis for a finite volume scheme. The steps involve integrating over the control volume $V_i$ (Equation 2-11), using the divergence theorem to convert the second term to a surface integral (Equation 2-12), and converting $\Phi$ to the volume-average $\bar{\Phi}$ (Equation 2-13):
\[
\iint\int_{V_i} \left( \frac{\partial \Phi}{\partial t} + \nabla \cdot F(\Phi) \right) dV = 0,
\]
2-11
\[
\iint\int_{V_i} \frac{\partial \Phi}{\partial t} dV + \iint_{S_i} (F(\Phi) \cdot n) dS = 0,
\]
2-12
\[
\frac{\partial \overline{\Phi}}{\partial t} + \frac{1}{V_i} \iint_{S_i} (F(\Phi) \cdot n) dS = 0.
\]
2-13

For 1D, \( n \) is a piecewise constant vector that points outward normal, and \( V_i = \Delta x_i \), such that Equation 2-13 becomes
\[
\frac{\partial \overline{\Phi}}{\partial t} + \frac{1}{x_i} \left( F(\Phi_{i+1/2}) - F(\Phi_{i-1/2}) \right) = 0.
\]
2-14

Converting the time derivative to a forward difference and solving for \( \overline{\Phi}^{k+1} \) yields the discretized equation
\[
\overline{\Phi}^{k+1} = \overline{\Phi}^k + \frac{\Delta t}{\Delta x_i} \left( F(\Phi_{i+1/2}) - F(\Phi_{i-1/2}) \right),
\]
2-15
which provides an explicit time-marching scheme this is the basis for many finite volume schemes. For the case of a single cell-type, \( \Phi \) and \( F(\Phi) \) become \( \phi \) and \( f(\phi) \), respectively.

 Appropriately approximating interface flux values \( F(\Phi) \) represents the major (and subtle) differences between finite volume integration schemes. This section does not attempt to review these methods, but rather to describe the method used in this work: Godunov’s method [51]. For simplicity, this discussion will restrict itself to the scalar, or 2-component case\(^1\). It treats each discontinuity in \( \phi(x, t) \) as a Riemann problem centered on the interface between grid cells. A Riemann problem is a conservation law with the discontinuous initial condition

---
\(^1\) The book “Finite Volume Methods for Hyperbolic Problems” by Randall LeVeque is a fantastic resource for understanding the Riemann problem for many components and dimensions.
where the wave speed on either side of the discontinuity is given initially by $f'(\phi_0)$. The solution to the Riemann problem is the value $\phi(x_o)$ just after the wave begins to move. The method is well suited for finite volume integration methods because each interface of the profile $\bar{\phi}(x, t)$ can be thought of as a Riemann problem, the solution to which provides the interface values for the fluxes in Equation 2-15. Godunov’s flux function provides the flux at a cell interface by treating the interface as a Riemann problem:

$$ f = \begin{cases} \min_{\phi_L \leq \theta \leq \phi_R} f(\theta), & \phi_L < \phi_R \\ \max_{\phi_R \leq \theta \leq \phi_L} f(\theta), & \phi_L > \phi_R \end{cases}. $$

This function is derived from a case-by-case analysis of Riemann problems. It generalizes the upwind scheme and gives the entropy-satisfying (a.k.a. physical) solution even for non-convex flux functions. Godunov’s method uses Equation 2-15 with the Godunov flux function (Equation 2-17) and is spatially 1st order accurate when using the piecewise-constant approximation $\bar{\phi}(x, t)$. Higher order approximations to $\phi(x, t)$ achieve better accuracy.
3 METHODS AND MATERIALS

3.1 Definitions and disk naming scheme

Creating some defining terminology helps to describe aspects of the separation process. These terms are briefly defined here. Spinup and spindown are, respectively, the brief acceleration and the 2-minute deceleration period that begins and ends a spin. Spindown is comprised of piece-wise constant, decreasing deceleration rates (see Wood et al. [52]). Figure 3 shows the anatomy of a disk. “Flowdown” is all the fluid (plasma-saline-red cell suspension) that enters the collection area at the end of a spin. Reported values for volumetric flowdown are what was actually collected from the disk and is called “plasma volume”. “Breakthrough” refers to the location(s) on the weir slope where the flowdown flows from the vestibule to the collection area. The “wetting front” is the location of the fluid-air interface on the weir. “Weir” refers to the divider between the collection area and the trough. The top of the weir is flat, and the term “weir slope” refers to the radially inward, curved face. The “trough” holds the red cells at the end of a spin. The “vestibule” is the empty space between the weir top and the lid that contains fluid when the disk is spinning.

Iterative disk design changes necessitated a descriptive naming scheme, so disk names describe important features: an example name “matte_20.05_16x3_16b_0.75x9mm_3mL_peo” is interpreted as follows:
Figure 3 Schematic of a disk, including design features. A blood sample goes into the collection area and the disk is accelerated. As the disk slows, red cells collect into the trough and plasma flows over the weir into the collection area. Ruffles are 3 v-shaped cuts into the weir slope to direct plasma breakthrough; baffles extend into the vestibule to prevent remixing; wells in the collection area reduce wetted surface area. The lid of the disk is omitted for clarity.

- “matte” describes the disk finish. If absent, the disk has a glossy finish.
- “20.05” refers to weir channels with half angle ($\alpha_{channel}$) of 20° and depth 0.05 inch.
- “16x3” means there are 16 ruffles, or groupings of 3 adjacent channels (each channel having a half-angle of 20° and being 0.05 inch deep). When this is absent, it means that the channels uniformly circle the weir. Ruffles extend radially 8mm (or 11mm if designated as “long”) into the collection area from the inner edge of the weir top.
- “16b” is the number of baffles. Baffles are partial walls aligned in the radial direction that extend onto the weir slope to encourage flowdown and reduce mixing.
during spindown. They are from previous work. When channels and ruffles existed in the same disk, they were evenly spaced apart (i.e. did not touch).

- “0.75x9mm” describes wells of depth 0.75mm and of radius 9mm cut into the collection area at the end of a channel. The number of wells matches the number of ruffles.
- “3mL” is the trough volume as measured from the bottom of the trough to the top of the weir. If absent, the default trough volume is 4 mL.
- “peo” refers to a dried film of PEO ($M_v = 100,000$) on the weir.

Dimensions and volumes of the trough and vestibule were measured using intersections in Solidworks. Table 5 in Appendix A shows selected dimensions.

### 3.2 Rapid prototyping

Rapid prototypes were 3D printed using a photocurable resin with a water-soluble support material. (Stratasys Objet30 Prime). The body of a printed object is a stiff, cross-linked acrylate, and printed features have a nominal resolution of 0.1 mm. This resin forms a smooth, translucent surface, and is referred to as "glossy". If the printed object has body material that cannot be directly supported by the body resin underneath, the printer uses a water-soluble support resin. Removing the support resin requires at least power washing, but more extensive removal of support requires soaking the whole object in aqueous 5-10 wt% NaOH solution for about 10 minutes. Even after extensive washing, support material can sometimes still be scratched off the body surface. Consequently, objects that have not been reproducibly cleaned contain residual support material. Any surface printed with the support materials is rough and opaque and is referred to as “matte”, regardless of how clean it is. Immersing a printed object in a solvent for
longer than an hour caused the object to swell asymmetrically. This meant that disks could not be soaked indefinitely to remove all support material from the trough.

3.3 Contact angle measurements

One-inch square wafers were printed with matte and glossy finishes. Glossy wafers simulated hydrophobic polymers used in injection molding, such as polypropylene. Matte wafers were printed to simulate the weir slopes of disks from previous work.

The contact angles of water and plasma were measured by placing a 2-4 μL drop on the wafer surface deposited with a 10 μL Hamilton syringe and photographed with a goniometer system (Sony Hyper HAD CCD-IRIS/RGB). Preliminary screening showed no convincing evidence that drop size affected contact angle in these experiments, so the drop volume was adjusted to maximize the drop size, while keeping the entire drop inside the camera field of view. Contact angles were measured using the “Contact Angle” plugin for imageJ (developed by Marco Brugnara).

Plasma proteins were coated onto the wafer surfaces to simulate the coating process that occurs when blood is spun in the disk. In one method, 200 μL of plasma was pipetted onto a wafer and removed after 1 minute. The remaining film was allowed to dry at room temperature for 5-10 minutes. This is denoted as “air.dry10min”. In the second treatment, 200 μL of plasma was pipetted onto a wafer over 20 seconds. Then, the wafer was blown on by a compressed air jet for 3 minutes (the approximate duration of a spinning experiment) to remove excess plasma and dry the wafer surface. This is referred to as “blown3min”. Third, a wafer was subjected to a strong air jet as 200 μL of plasma was pipetted onto the surface along the edge. The plasma immediately blew across the wafer, and the wafer stayed in the air jet for 1 minute. This is
referred to as “quick.dry”. After taking contact angle measurements on a coated wafer, the wafer was scrubbed by hand with a wet Kimwipe and allowed to air dry. This is denoted as “wiped.”

3.4 Wafer experiments

The possibility of directing flowdown to specific locations is appealing because it gives greater flexibility in designing future disks. Uniform flowdown is also desirable because it distributes shear rate on the red cell pack around the whole disk, leading to less red cell entrainment in the recovered plasma or essentially less red cell recovery. With this in mind, we wanted to see if cutting v-shaped channels could create a wetted path for bulk plasma flow. Due to the complex geometry of the disk weir, it was desirable to test the effect of channels in a simplified scheme. The weir slope was approximated using 1 x 1 x 0.1 inch wafers printed with v-shaped cutouts along 3/4 of the largest surface, as shown in Figure 4. A 0.25 x 1 inch region was left bare to act as a reservoir. The reservoir and channels were separated by a v-shaped notch perpendicular to the channels. The purpose of this notch was to prevent liquid contact with the channels while reservoir was being filled. The depth and angle of this notch were the same for all wafers printed. Six wafers were printed with channel half-angles of 20, 50, and 70° and depths of 0.025, and 0.05 inch. Two wafers were also printed without channels to serve as controls. The first contained only the notch, and the second had a flat cut 0.05 inch in depth. Each wafer's ID is the concatenation of the channel half angle and the depth. For example, a wafer with 20° channels cut to 0.025 inch is referred to as "20.025". Wafers were printed with matte and glossy finishes (as described above) and cleaned before use.
Figure 4 Example of a test wafer. Fluid was placed on the reservoir and tilted to cause flow into the channels. The angle at which the fluid moved showed how suitable the channel was at inducing flow. This wafer has a half-angle of 50° and depth of 0.05 inch.

The apparatus for measuring inclination angle consisted of a protractor and horizontal flat plate that could be inclined by hand. To do an experiment, a wafer was placed onto the plate with the reservoir furthest away from the axis of inclination. The reservoir surface was filled with either plasma or water. After the reservoir was wetted, the notch and the tips of the channels were also wetted, and a total of 250 μL was added to the reservoir. The plate was slowly inclined, and the angle was recorded at which the liquid front moved at a minimal, but constant rate. In some experiments, the liquid front moved abruptly. In this case, 1 of 2 approaches was taken: (1) If the liquid ran quickly down the channels and off the wafer, the angle at which it rolled was measured. This occurred more frequently on uncoated wafers. (2) If the plate could be declined fast enough to "catch" the drop before it rolled off the wafer, then the plate was slowly re-inclined to attempt a constant liquid speed. Often, this circumstance required a few iterations to obtain a constant liquid speed. Once the angle was measured, the wafer was dried with a paper towel and with compressed air for at least 30 seconds.

2 Higher volumes than 250 μL caused the liquid front to advance before the plate was inclined.
3.4.1 Polymer coatings

To test the usefulness of hydrophilizing the weir with a polymer coating, the wafers were independently coated with poly(acrylic acid) (PAA, Sigma-Aldrich, $M_v = 450,000$) and poly(ethylene oxide) (PEO, Sigma-Aldrich, $M_v = 100,000$) dissolved in methanol. PEO is only lightly soluble in methanol (0.4 wt %), but more soluble when heated (3.4 wt % above 45 °C). The wafer channels were coated with either a PAA or PEO solution using a foam brush. The reservoir was not coated. Coated wafers were left to dry at room temperature for 10 min to 4 hours before measuring roll-down angles.

The inclination procedure of Section 3.4 was done with slight modification. After the angle was measured, the water was spread along the channels using the side of the pipette tip and left there for at least 10 seconds. Then, the wafer was dried for at least 30 s in compressed air and no paper towel. This is referred to as 1 cycle. Subsequent cycles followed immediately.

3.5 Spinning experiments

3.5.1 Disk preparation and spinning

The support material had to be removed from the disks before use. For the wide, flat surfaces of the disk, much of the support material could be scraped off. The trough and weir were cleaned using a water jet at high pressure. This removed the bulk of the support but did not remove support material on the disk surfaces. Matte disks were cleaned no further than this.

3 A brief investigation of solvents was done to screen for maximum volatility and minimal interaction with the disk material. It was found that methylene chloride (NBP = 39.6 °C) and ethylene dichloride (NBP = 83.47 °C) extracted low MW polymer from the disk material and a poly(propylene) (PP) sample. Acetone was found to asymmetrically swell the printed material, leading to rotational instability. Eventually, methanol (NBP = 56 °C) was used because it interacted with neither the printer material nor PP.
Glossy disks were cleaned further by filling the trough with a 5-10 wt% sodium hydroxide solution for at least 10 minutes before re-spraying the trough with the water jet.

Residual support in the trough was problematic because it artifactually reduced the total volume ~80-85% of the nominal trough volume for glossy disks. The real trough volume was tested by placing the disk on a level surface and filling the trough until all the inner trough wall was wetted. Once the disk is soaked in NaOH solution, the real volume increases to ~90% of the nominal designed volume. This difference between real and nominal volume was not explicitly tested for matte disks. However, the volume difference should be about twice as large in matte disks because both the inner and the outer faces of the trough are printed with support material. In addition, the trough held 10-20% more than its nominal value (by of a meniscus) before the liquid began to flow down the weir.

Each disk was prepared for spinning by rinsing it with tap water, drying off the excess liquid with a paper towel, and using compressed air to remove water from the trough area. The disk was secured onto a motor platform. After that, 7 mL of whole blood and 1.5 mL saline were pipetted into collection area. These same volumes were used in all experiments unless otherwise stated. The disk accelerated by 500 revolutions per minute (RPM) each second (RPM/s) until 3000 RPM, held that speed for 54 s (unless otherwise indicated), and then decelerated over ~2 minutes. Alizadeh et al. give a detailed spinning velocity profile [14]. Once the disk stopped, the flowdown plasma was collected, weighed, and measured for hematocrit. Blood collected from

---

4 The printer can only print a glossy surface when there is no overhanging body. Previous (and continuing) work had body material hanging over the inner edge of the trough, so this face had support material that needed to be removed. Additionally, the disk lid hangs over the trough and weir, so glossy disks were printed without their lid, and the lid was glued on after cleaning out the trough.
anonymous donors was refrigerated for no more than 24 hours and warmed to room temperature before spinning.

### 3.5.2 Bacteria preparation and counting

Bacteria (*E. coli*: strain: BL21 DE3 star) were grown in nutrient broth overnight from a streaked plate. The suspension was pelleted and resuspended in phosphate-buffered saline (1xPBS) to give concentrations between $10^4$ - $10^6$ colony forming units (cfu) per mL. In experiments using bacteria, 100 µL of this suspension (or 65 µL for dilution experiments where the blood was diluted to hematocrit of 0.05 and 0.21) was spiked into equal volumes of PBS (control) and PBS-diluted blood. The disk was filled with enough of this spiked blood mixture to give a consistent fluid thickness of 3.3 mm. Table 5 (in Appendix A) shows the volumes of whole blood-PBS suspension spun in the disk.

The collected plasma and the spiked PBS were diluted 1:9 in PBS and 50 µL of dilutions $10^{-2}$ through $10^{-5}$ were plated. Plates were incubated at 47 °C for 16+ hours and counted by hand. Bacterial concentration recovery (see Section 3.5.3) was the ratio of bacterial concentrations in a plasma sample divided by the average of bacterial concentration in the spiked PBS (called control) for that set of experiments.

### 3.5.3 Definition of plasma, red cell and bacteria recoveries

Disk designs were evaluated by the fraction of the total plasma or red cells that flowed into the collection area at the end of a spin. This section derives plasma, red cell, and bacteria recovery from volume balances. Experiments that focused on disk designs did not use bacteria, so plasma recovery is a surrogate value for bacteria recovery. This derivation is more precise
than previously used by Pitt et al. [14] because it accounts for flowdown hematocrit and plasma-PBS solution density.

The first step is to convert flowdown mass to volume. The flowdown is treated as a binary mixture of red cells and plasma-saline solution. Assuming a constant $\rho_{\text{plasma}} = 1024 \text{ kg/m}^3$ and $\rho_{\text{PBS}} = 1006 \text{ kg/m}^3$, the plasma-saline solution density $\rho_{\text{solution}}$ can be solved for:

$$m_{\text{solution}} = \rho_{\text{PBS}}V_{\text{PBS}} + \rho_{\text{plasma}}(1 - HCT_{\text{whole blood}})V_{\text{whole blood}}, \quad 3-1$$

$$\rho_{\text{solution}} = \frac{m_{\text{solution}}}{V_{\text{whole blood}}(1 - HCT_{\text{whole blood}}) + V_{\text{PBS}}}, \quad 3-2$$

where $V_{\text{whole blood}}$ and $V_{\text{PBS}}$ are controlled. The symbol $HCT$ designates experimental hematocrit values instead $\phi$ for theoretical calculations. $HCT_{\text{whole blood}}$ is the hematocrit of the donor’s blood. The volume fraction of plasma in this solution is

$$\nu_{\text{plasma}} = \frac{V_{\text{whole blood}}(1 - HCT_{\text{whole blood}})}{V_{\text{PBS}} + V_{\text{whole blood}}(1 - HCT_{\text{whole blood}})}. \quad 3-3$$

With $\rho_{\text{solution}}$ known, the mass of the flowdown is given by

$$m_{\text{flowdown}} = \rho_{\text{solution}}V_{\text{solution}} + \rho_{\text{RBC}}V_{\text{RBC}}, \quad 3-4$$

Moving to the next step requires the intermediate values: $V_{\text{solution}} = V_{\text{flowdown}}(1 - HCT_{\text{flowdown}})$ and $V_{\text{RBC}} = V_{\text{flowdown}}HCT_{\text{flowdown}}$. Substituting these values into the equation above yields:

$$m_{\text{flowdown}} = V_{\text{flowdown}}\left[\rho_{\text{solution}}(1 - HCT_{\text{flowdown}}) + \rho_{\text{RBC}}HCT_{\text{flowdown}}\right], \quad 3-5$$

and a simple rearrangement of Equation 3-5 gives $V_{\text{flowdown}}$.

---

\[ V_{flowdown} = \frac{m_{flowdown}}{\rho_{solution}(1 - HCT_{flowdown}) + \rho_{RBC}HCT_{flowdown}}. \] 3-6

Now the plasma recovery can be calculated:

\[ Plasma\ Recovery = \frac{\nu_{plasma}V_{flowdown}(1 - HCT_{flowdown})}{V_{whole\ blood}(1 - HCT_{whole\ blood})}, \] 3-7

which, after cancelling some terms associated with \( \nu_{plasma} \), simplifies to the final form:

\[ Plasma\ Recovery = \frac{V_{flowdown}(1 - HCT_{flowdown})}{V_{PBS} + V_{whole\ blood}(1 - HCT_{whole\ blood})}. \] 3-8

This definition of plasma recovery tracks the amount of original plasma that the disk recovers, regardless of any amount of dilution with PBS. This keeps the metric in terms of the original whole blood sample.

Red cell recovery follows a similar volume balance:

\[ RBC\ Recovery = \frac{V_{flowdown}HCT_{flowdown}}{V_{whole\ blood}HCT_{whole\ blood}}. \] 3-9

For experiments involving bacteria, “concentration recovery” is the bacterial concentration in the recovered plasma divided by the concentration in the blood before spinning (as measured by the control: see Section 3.5.2):

\[ Concentration\ recovery = \frac{C_{b,flowdown}}{C_{b,0}}. \] 3-10

Here, \( C_{b,0} \) is the concentration of bacteria in the diluted whole blood instead of in the original whole blood. Bacteria recovery is the number of bacteria in the collected plasma divided by the number of bacteria in the disk before spinning, as measured by the saline control:

\[ Bacteria\ recovery = \frac{C_{b,flowdown}}{C_{b,0}} \frac{V_{flowdown}}{V_{whole\ blood} + V_{PBS}}. \] 3-11
3.6 Statistics

Statistical hypothesis tests were chosen for how appropriately the data met the model assumptions. Two-sample or paired t-tests were used when applicable, but non-parametric rank tests were used when these assumptions were not satisfied, such as for RBC recovery where values may not decrease below 0. Data and code for all statistical analyses can be found in the “data-analysis” folder in the github repository (https://github.com/cliffanderson720/MS-Thesis-Pitt). Statistical analysis and figures involving experimental data were done using the R programming language.

A note on experimental design: surprising variability exists between blood samples from different donors. See Section 5.2. However, blocking and randomization of blood samples for most disk design experiments were haphazard or nonexistent for early experiments, unless otherwise specified. In these cases, efforts at blocking and randomization are detailed.

Experimental values are reported as mean and standard deviation unless otherwise noted. Regressions are fitted using a least-squares procedure, and points were discarded if their Studentized residuals exceeded 2.
4 EXPERIMENTAL RESULTS AND DISCUSSION OF DISK DESIGNS

This chapter describes disk modifications and their effects on breakthrough, plasma recovery, and red cell recovery. It first describes general surface properties of the disk material, followed by exploratory experiments aimed at inducing capillary flow. The remainder of the chapter focuses on disk modifications that were informed by past work and ongoing findings. Specific recommendations are postponed until Chapter 6.

4.1 Contact angle

We observed that disk material wetted more easily after it had been coated with a film of dried blood. Wettability is desirable, but the extent to which the weir surface was affected by contact with the blood was unknown. Vroman showed that small proteins, such as antibodies, adhere rapidly to surfaces and are eventually displaced by proteins with greater affinity for the surface [53]. The affinity of blood proteins is strong enough that solvents alone cannot remove them [54]. Furthermore, studies on plasma protein adsorption onto polymeric surfaces suggest that adsorbing proteins such as albumin, globulin, fibrinogen, or IgG orient their most polar regions toward a polar surface [55]. Consequently, the more hydrophobic regions of a protein orient toward the disk surface, leaving hydrophilic ends oriented towards the air, which makes the surface more wettable to water. In this section, we investigated the effect of plasma exposure
on disk material wettability by measuring the contact angles of water and plasma on glossy and matte surfaces coated by human plasma.

The contact angles measured on glossy wafers are displayed in Table 1. On a clean glossy surface, water had a greater contact angle than plasma (64.1±4.5° and 53.9±6° respectively, \( p = 0.04 \)) because of water’s polarity. The treatment “air.dry10min” left a thick coating, however, which decreased contact angles for water and plasma as each droplet solvated the plasma coating.

<table>
<thead>
<tr>
<th>Material(^1)</th>
<th>Liquid</th>
<th>Coating Type(^2)</th>
<th>Contact Angle ((\theta_f))</th>
<th>SD</th>
<th>n</th>
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</thead>
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<td>clean</td>
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<td>6.5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>clean</td>
<td>53.9</td>
<td>6.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wiped</td>
<td>67.3</td>
<td>3.8</td>
<td>16</td>
</tr>
<tr>
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<td>plasma</td>
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<td>5.3</td>
<td>27</td>
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<td></td>
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<td>3.9</td>
<td>4</td>
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<td></td>
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<td>4</td>
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<td></td>
<td></td>
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<td>2.1</td>
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<td>clean</td>
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<td>6.2</td>
<td>43</td>
</tr>
<tr>
<td></td>
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<td>64.1</td>
<td>4.5</td>
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<td>5.6</td>
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<td>91.3</td>
<td>10.9</td>
<td>4</td>
</tr>
</tbody>
</table>

\(1\) Wafers made of polypropylene (PP) and 3D-printed acrylic with either a matte or a glossy surface

\(2\) Refers to whether the wafers had a coating. Contact angle, standard deviation, and replicates are based on pooled data from Treatments.

\(3\) Refers to how that coating was applied (see Section 3.3)
To more accurately replicate the short plasma-surface contact time in a spinning experiment, treatment “blown3min” was applied and found to maintain a low contact angle (28.1°) for two repetitions. Removing the dried plasma film with a wet Kimwipe raised the contact angles of water (71.1±6.2°) and plasma (53.9±6°) above those on the original clean surface ($p < 0.05$ for two-sample t-test between “clean” and “wiped” for both plasma and water), indicating that the plasma coating may be removed from a disk through mechanical abrasion. Treatment “blown3min” gave a qualitatively thinner coating than “air.dry10min” but seemed to over-estimate the contact time experienced in an actual spinning experiment. Treatment “quick.dry” did not leave a film of visible thickness, but its contact angle remained low (23.4±3.9°). This indicates that short contact times with plasma are sufficient to increase the wettability of a hydrophobic surface.

One goal of this study is to find how dilution affects the separation process (See Chapter 5). To test saline’s effect on wettability, contact angles of diluted plasma were measured on a clean surface and on a surface with a “quick.dry” coating of diluted plasma. The plasma coating and the droplets of plasma came from the same plasma solutions. Figure 5 shows contact angle versus volume fraction of plasma. On a clean surface, contact angles appear roughly equivalent for all dilutions (ANOVA $p = 0.055$). However, on a coated surface, contact angle is low and seems to decrease with plasma dilution, even for the 1:9 plasma:PBS dilutions. This suggests that a relatively low concentration of plasma still has enough proteins to coat a surface, even for the very short contact time of the “quick.dry” treatment. So, blood dilution prior to spinning should not hinder breakthrough but may in fact encourage wetting.

---

6 Diluents should not lyse red cells because heme in the lysate interferes with downstream genotypic tests. 1xPBS keeps red cells intact.
Figure 5 Contact angle of plasma-saline dilutions on plasma-coated 3D printed wafers. Wafers coated with diluted plasma (“quick.dry” treatment) are very hydrophilic, even when plasma is diluted 1:9 with saline.

Uniform flowdown after deceleration may return clearer plasma by reducing shear at the plasma-cell pack interface. During deceleration, the plasma is pulled down by gravity, but is prevented from advancing down the weir by the work required to wet the weir surface. Instead, the liquid bulges near the wetting line until breakthrough, where increasing hydrostatic pressure finally overcomes the wetting resistance. Breakthrough can be abrupt and entrain red cells from the sediment layer. Often, the plasma flows down the weir slope in 1 to 3 locations, providing a wetted surface for the rest of the plasma to flow over down into the collecting area. It’s possible that a hydrophilic weir surface may present a smaller surface energy barrier, permitting more uniform flowdown and less entrainment of RBCs. The data in Figure 5 also indicate that a plasma coating does not affect wettability for matte as much as for glossy. Glossy surfaces are preferable because the data statistically verified that they had more uniform flowdown and faster breakthrough (see Section 4.3.1).
4.2 Liquids on tilted wafers

To test the effects on breakthrough that might be caused by cutting channels into the weir, we prototyped wafers with v-shaped channels of varied half-angles ($\alpha_{channel} \in [20,50,70^\circ]$) and depths (0.0, 0.025, 0.05 inch) and measured how much the wafer needed to tilt before the liquid would flow down the surface. Each wafer's ID is the concatenation of the channel half angle and the depth. Inclination angles for water on glossy wafers coated with PEO are shown in Figure 6. The inclination angle was the lowest for freshly PEO-coated wafers, and some channels wetted spontaneously\(^7\). The inclination angle reached a steady value with repeated cycles as the PEO washed off. Final inclination angles decreased with channel half-angle for 0.025 inch channels, indicating that narrow channels provide less resistance to wetting. Narrower channels also took longer to reach their final angle, suggesting that narrow channels retained the PEO better.

![Figure 6 Summary of PEO coating effect on inclination angles for consecutive cycles (n=4, SE shown). Each wafer with the PEO coating wetted easily. As the experiment was repeated, the polymer washed off, and the angle at which the fluid steadily moved down the channels increased to a steady value. The hydrophilic PEO coating washed off for all wafers except 20.05.](image)

\(^7\) A few experiments using plasma as the liquid also wetted spontaneously
At a depth of 0.05 inch, this trend is less clear. What is of special note, however, is that the wafer 20.05 wicked water from the reservoir down the channels for every cycle. Due to the sharp wedge geometry, perhaps solvated PEO remained in the channels’ bottoms despite the water removal after each cycle. This means that the 20.05 channels reliably wick water, even if the hydrophilic coating is compromised. The 20.025 wafer also wicked water down the channels, but these inclination angle experiments only measured bulk flow down the wafer. This was intentional because when breakthrough happens on a disk, the plasma flows down in bulk rather than purely by capillary flow.

In order to understand these results, Berthier gives an inequality to predict spontaneous capillary flow in v-shaped open channels (Equation 4-1) (see pg 40 Table 1.1 of ref [56]):

\[
\frac{w}{2d} < \cos \theta_{fs}.
\]  

(4-1)

Here, \(w\) is the peak-to-peak channel width, \(d\) is slant height, or trough-to-peak distance, and \(\theta_{fs}\) is the fluid-solid contact angle. Spontaneous capillary flows occur when this inequality holds. Using the identity \(\frac{w}{2d} = \sin \alpha_{channel}\), Equation 4-1 becomes

\[
\sin \alpha_{channel} < \cos \theta_{fs},
\]  

(4-2)

where \(\alpha_{channel}\) is the channel half-angle. The same inequality in slightly less trigonometric form reads:

\[
90 - \alpha_{channel} < \theta_{fs}, \quad \alpha_{channel} \in [0,90].
\]  

(4-3)

According to this theory, a contact angle lower than 70° is sufficient to cause SCF in a 20° channel. Thus, broad channels may induce SCF for an extremely wettable surface (i.e. \(\theta_{fs}\) tends
to 0°) but may not induce anything if the surface becomes less wettable. This theory supports the wicking observed in 20° channels and can guide future work in creating capillary flow.

4.3 Disk weir design

This section defines disk features and describes weir modifications and their effect on breakthrough. Specific effects on plasma and red cell recoveries are discussed in Sections 4.5 and 4.6. The reader is encouraged to review disk features shown in Figure 3 before continuing.

4.3.1 Glossy

Replacing the matte finish with a glossy finish reduces the within-disk standard deviation of volumetric flowdown by 67% (54-76 95% confidence, $F_{0.975,128,29}$). Plasma-coated glossy and matte surfaces have similar contact angles (22.7°±3.3 vs 28.5°±5.6 $p = 0.053$), so contact angle alone does not explain the difference. Rather, it may be the presence of unremoved support material. Support material can be easily removed from a flat surface, but weir slopes and modifications, such as ruffle and baffles, are difficult to clean.

4.3.2 Ruffles

The capillary flow results from the 20.05 wafer suggested that channels cut into the weir could induce breakthrough in predetermined spots. Disks were printed with 16 ruffles, or groupings of 3 contiguous channels, cut into the weir to induce breakthrough by capillary flow. As a disk decelerated below ~100 RPM, beads of plasma initiated by the ruffles appear on the top weir edge and usually broke through in 2-7 out of 16 places (See Figure 8 for an example).
16 ruffles were chosen to evenly compare to disks containing 16 baffles, but the breakthrough occurs qualitatively the same for disks with 8 ruffles\(^8\).

On disks with 16 ruffles, the ruffles were cut to full depth (0.05 inch) at the weir top, but they grew shallower as they descended into the collection area. This was problematic because shallow channels do not wet as easily as deeper ones (See Section 4.2). On disks with 8 ruffles, we extended the ruffles further into the collection area (from 8 mm to 11 mm), which allowed the channels to cut deeply into the weir for a greater distance along the weir slope before tapering off. Figure 7 shows breakthrough points, or the number of locations where ruffles brought plasma all the way into the collection area. Long ruffles are deeper for longer and appeared to induce breakthrough more consistently (rank-sum test \(p = 0.102\)). Having consistent breakthrough means that plasma flow can be controlled, which presents an opportunity for future automation.

![Breakthrough points](image)

**Figure 7** Breakthrough points associated with short and long ruffles. Longer ruffles induced breakthrough more reliably.

\(^8\) Additionally, we observed that the number of breakthrough points increased with decreasing hematocrit.
The spontaneous capillary flow (SCF) theory does not explain everything though. Matte contact angles for plasma-coated surfaces ($\theta_{fs} = 28.5^\circ$) predict capillary flow for $\phi_{channel} < 61.5^\circ$. However, SCF never occurred on breakthrough for any matte disks with ruffles.\(^9\)

### 4.3.3 Wells

The plasma film that remains on a glossy surface after pipetting up the bulk fluid has a mass of 3.72 mg/cm\(^2\) (±0.23 95% confidence) of plasma-wetted surface. On an average spin, the wetted area is about 25 cm\(^2\), so about 0.1g (up to 5% of the total plasma) of the flowdown is unrecoverable by pipetting. We reduced wetted surface area in a disk by cutting wells into the collection area so that plasma could bead up. For these experiments, breakthrough occurred solely into the wells for 32 out of 34 experimental spins. The ruffles generated breakthrough and the wells controlled where plasma flowed. Less reliable, however, were the number of breakthrough points and of wells that actually filled up with plasma. A typical spin had 3-7 breakthrough points, and 1-3 fully filled wells. Diluted blood, however, consistently had 8 evenly filled wells, although none were full. This suggests that the breakthrough location of diluted blood may be easier to direct, perhaps because of lower viscosity or higher surface tension.

Examples of flowdown on each type of disk are shown in Figure 8.

\(^9\) Residual support material in the channels did not always dry completely between spins. Blood in the collection area in preparation for the next spin occasionally wicked up the channels. This was fun to watch.
Figure 8 Photographs showing breakthrough on different disks. The left photograph shows a disk with wells (disk: 20.05_8x3_1.25x7mm_H5) partially filled with flowdown. Diluted blood filled these wells evenly. The middle photograph shows a top-down view of glossy disk (disk: 16b) where the breakthrough occurred uniformly around the weir. The middle and right photographs show red cell entrainment to various degrees from ruffles and baffles. The top right photograph shows red cell entrainment on a matte disk with ruffles and baffles (disk: matte_70.05_16x3_16b). The bottom right photograph shows various stages of breakthrough and entrainment due to ruffles on a glossy disk (disk: 20.05_16x3).

4.4 Flowdown volume

Flowdown volume is closely related to plasma recovery, but it is an un-normalized metric for recovered fluid. Flowdown volume is important for using the mathematical model because it gives estimates of the cutoff in the $r$-$t$ plane (see Chapter 5). Figure 9 shows the volumetric flowdown for each disk. The flowdown values for matte disks are likely biased high because excess support material in the trough decreases its available volume (see Section 3.5.1).

4.5 Plasma recovery

Plasma recovery describes the percentage of plasma from the ingoing blood that is collected at the end of the spin. It normalizes for input whole blood volume and hematocrit and serves as a surrogate for bacteria recovery for experimenting on disk designs. Plasma recoveries for all disks are shown in Figure 10 (also in Appendix A).
Figure 9 Volumetric flowdown for all disks. Feature modifications are grouped by type, and individual disk names are given on the y axis. Means for each disk are shown as a black vertical line. Glossy and matte disks are represented by ○ and ●, respectively. The group “Base” has an unmodified weir and serves as the control. All modifications improved plasma recovery above the base design. Wide weirs and matte surfaces increase variability. In all experiments, 7 mL of whole blood and 1.5 mL of PBS were placed in the disk.

4.5.1 Weir width

The weir is the divider between the red cell trough and the collection area. Previous disks made by Alizadeh et al. used a weir top 1.3 mm wide. However, cutting ruffles into the weir required that the weir top be wider than 1.27 mm (0.05 inch). Thus, the weir was extended radially inward until 2mm wide for all disks except “16b” and “base.” The weir width is a confounding factor between the base design and all other modifications. To bridge the gap, we spun blood on the base design with both 1.3 mm and 2 mm weir widths. The “Base” section of Figure 10 shows plasma recoveries on both disks. Increasing the weir top width decreased
Figure 10 Plasma Recovery for all disks. Feature modifications are grouped by type, and individual disk names are given on the y axis. Means for each disk are shown as a black vertical line. Glossy and matte disks are represented by ○ and ●, respectively. The group “Base” has an unmodified weir and serves as the control. Modifying the weir can greatly increase plasma recovery.

plasma recovery by 0.169 fractional units (±0.097 unequal variances t-test with d.f. = 12.8 at 95% confidence). In addition, the disk base_2mm_weir delayed flowdown up to 60 seconds after the disk stopped moving because the dry weir edge hindered wetting of the weir slope where breakthrough happens. The inherent uncertainty in delayed breakthrough is a great source of variation in final flowdown volume. The standard deviation of plasma recovery in base_2mm_weir is 1.89-fold (1.02-3.51 \(F_{0.975,9,12}\)) greater than for the base disk with a 1.3 mm weir. One reason for this is that when breakthrough only happens in one or two locations of the disk, plasma in the vestibule on the other side of the disk does not migrate over to the
breakthrough point to flow down. The longer breakthrough takes to happen, the more likely it is that it will only happen in one place, and hence that there will be more unrecovered plasma.

Even in disks having consistent breakthrough, the trough/vestibule area holds more fluid than the designed trough volume (see Section 3.5.1). A brief experiment indicates this volume is 0.88 mL (±0.15 95% confidence) for a 1:9 plasma:PBS solution on a disk with a shallow trough (volume: 0.37 mL, disk: 20.05_8x3_1.25x7mm_H5). Observations show that a meniscus forms between the weir top and the back wall. Changing the weir width tests the hypothesis that the unrecovered fluid is comprised of the meniscus. Making the weir wider should increase the volume of the meniscus (assuming that wetting angle is constant with geometry), trapping more plasma in the vestibule and reducing plasma recovery. The decreased plasma recovery associated with the wider weir supports this hypothesis. Future work could focus on reducing the meniscus volume because it constitutes up to 30% of the original plasma volume.

4.5.2 Effect of weir slope designs

Figure 10 shows plasma recovery for all disks, grouped by modification type. A clear difference exists between disks with modified and unmodified weir slopes. Specifically, the presence of baffles on the base disk increased plasma recovery by 0.164 fractional units (±0.043 95% confidence) more than for base. Ruffles and wells increased plasma recovery by 0.294 fractional units (±0.092 95% confidence) and 0.314 fractional units (±0.093 95% confidence) more than base_2mm_weir, respectively. Adding ruffles (and wells) to the 2 mm weir provides a pre-wetted breakthrough point for the plasma and reduces variability in plasma recovery. Although ruffles and wells improved plasma recovery much more than baffles did, they also
required a wider weir top, which by itself, performed worse than the base disk with a narrow weir.

Instead of viewing the marginal benefit of each modification type, they should be compared head-to-head because the base designs are not the same. An ANOVA among glossy disks grouped by ruffles, baffles, and wells suggests a difference between these disk types \( p = 6 \cdot 10^{-5} \) when excluding disks 20.035_8x3_1.25x7mm, 20.05_16x3_3mL, and 20.05_16x3_5mL for more direct comparison of weir modifications. \( p = 6 \cdot 10^{-4} \) when these disks are included. Tukey’s HSD\(^{10}\) suggests that baffles and wells, respectively, recover 0.040 (±0.022 95% confidence) and 0.020 (±0.018 95% confidence) more plasma than plain ruffles. These differences, however, are small and may take second priority behind ease of manufacturing or red cell recovery. In short, plasma recovery is sensitive to weir modification, but less sensitive to the specific type of modification.

As a note, some matte plasma recovery values appear surprisingly low given their high volumetric flowdown (Figure 9). This is from a small set of incomplete experiments in which the whole blood hematocrit was not recorded. Thus, volumetric flowdown could be estimated, but plasma recovery could not. The replicates with missing plasma recovery also happened to have very high volumetric flowdown.

4.5.3 Effect of collection time

After initial breakthrough, flowdown can continue for up to 3 minutes. When should the plasma be collected? At short times, the plasma has not wetted much surface area, so little plasma should be lost to the surface. However, collecting too early may preclude getting all the

\(^{10}\) Tukey’s Honest Significant Difference. A post hoc test with corrections for multiple comparisons.
recoverable plasma. The inverse may be true for a long delay in collecting the flowdown. Experiments in which flowdown was collected for 60 s following a delay of 0, 30, and 60 s after the disk stopped gave no convincing evidence that delay time makes a difference. Further tests are unlikely to reveal a trend. This means that users of the disk need not be too concerned about how long they wait to collect the flowdown.

4.6 Red cell recovery

This section summarizes red cell recovery over all disks. The next sections detail specific design effects. Figure 11 shows red cell recovery for modification categories as a function of whole blood hematocrit. Looking at the Ruffles category, the trough contains red cells up to a limit, above which red cell recovery increases with hematocrit. This trend is not as clear with other modification types. It may be noted that for $HCT_{whole\,blood} < 0.45$, the disk with ruffles has the lowest average red cell recovery.

Figure 11 Red cell recovery versus whole blood hematocrit for feature categories. Blood was diluted in the ratio 1.5mL PBS / 7mL whole blood. For Ruffled disks, the trough contains red cells up to a limit, above which red cell recovery increases with hematocrit.
4.6.1 Trough volume

The trough volume of 4 mL was designed to hold all red cells for $HCT \leq 0.57$ (4 mL/7 mL) assuming the red cells are fully packed to $HCT = 1$. Samples taken from the bottom of the trough after a spin at 3000 RPM for 54 s gave $HCT = 0.855 \pm 0.015$ (95% confidence). Using this value of $HCT$ in the cell pack, a 4 mL trough should accommodate $HCT > 0.486$.

However, red cell flowdown still occurs despite predicted excess trough volume. As the disk slows, the body force vector rotates smoothly downward and decreases from 600xg to 1xg over ~2 minutes. The denser red cell sediment then is thought to slide from the outer wall down into the trough. How do red cell recoveries change with trough depth? The trough depth was varied (at the same width) to hold 3, 4 and 5 mL. Each disk was spun with 6, 7 and 8 mL of whole blood/PBS at a same dilution ratio (1.5 mL/7 mL) to see if the trough depth quantitatively changed red cell recovery. Figure 12 shows red cell recoveries from an overlaid linear model $[HCT|RR \times Disk]$. Red cell recovery stays at 0 until a threshold hematocrit, after which red cell recovery increases linearly with hematocrit. The threshold hematocrits are predicted from the regression intercepts. They increase from 0.449 (±0.014 95% confidence) at 3 mL to 0.492 (±0.025 95% conf) at 5 mL troughs. The x-intercept increases with hematocrit and at 5 mL approaches the hypothetical threshold 0.486 calculated using the experimental trough packing factor (0.855). The red cell recovery rate also decreases between 3 mL and 5 mL from 1.50 RBC/HCT (±0.25 95% confidence) to 0.94 RBC/HCT (±0.21 95% confidence). The lower red cell recovery rate for 5 mL means that deeper troughs contain red cells better than shallow ones do.

There is a transition region around $HCT = 0.46$ for the 4 mL disk where red cell recovery is not linear. This suggests a fuzzy interface between plasma and red cells after spindown. PEO
coatings on the 4mL disk did not change the red cell recovery rate because the plasma film that covers or mixes with the PEO is also hydrophilic.

Figure 12 Red cell recovery versus hematocrit for trough volumes of 3, 4 and 5 mL. Above a threshold hematocrit, the trough ceases to fully contain the red cells, and red cell recovery increases linearly with hematocrit. The rate at which red cells come down decreases at the trough is deepened. Colored lines represent the linear model for data with red cell recovery above 0.0075 (dashed grey line).

4.6.2 Weir width

Weir width had no detectable effect on red cell recovery (rank-sum test \( p = 0.74 \)) for a glossy surface. That may mean that the shear on the red cell pack is roughly independent of trough-to-weir-edge distance. A confounding effect in this weir width comparison is that the slope of the weir is not wetted for the disk base_2mm_weir. This is problematic because breakthrough is abrupt if the slope is not wetted, and so weir edge distance is not the only factor that affects breakthrough. Overall, the optimal designs need to keep the path of the plasma away from the red cells in the trough (see Section 4.6.2). The slope modifications all reach close to the cell pack: baffles touch the back wall and ruffles cut as close as 0.75 mm to the edge of the trough.
4.6.3 Baffles

Pitt and McClellan introduced baffles in previous efforts to prevent remixing on spindown and to initiate breakthrough [14]. Figure 13 shows red cell recoveries for 3 pairs of baffled and unmodified disks. Baffles increased red cell recovery for spins using similar hematocrits (rank-sum test $p = 0.00001$, $n=39$). Baffles extend into the cell pack, unlike ruffles, so when baffles initiate breakthrough, downcoming fluid shears the cell pack near the baffle and entrains red cells into the collection area. Though the disk 16b has the highest average plasma recovery, it suffers because it has high red cell recovery relative to other disks at similar hematocrit (see Figure 14).

![Figure 13 Red cell recovery for 3 pairs of baffled disks and unmodified (i.e. base) disks. Paired disks are the same except for the presence of baffles. Over ranges of similar hematocrit, baffles increase red cell recovery because they extend into the red cell pack.](image-url)
Figure 14 Red cell recovery vs plasma recovery grouped by disk type. Data is shown for hematocrits between 0.45 and 0.5 on glossy disks with a 4 mL trough.

4.6.4 Ruffles

One disadvantage to using ruffles is that they cut close to the trough, so the entrance to the channels is only about 0.75 mm away from the red cell pack in the trough. As a result, the bottom of the ruffles tends to fill with red cells (see Figure 8) and bring them down into the collection area\textsuperscript{11}. To test the effect of channel depth on red cell recovery, the depth of the channel at the top of the weir was reduced from 0.05 inch to 0.035 inch (i.e. 0.035\_8x3\_1.25x7\text{mm}). The channel depth 0.035 inch was chosen because 0.025-inch channels did not induce bulk flow (see Section 4.2). The disks were spun at a range of hematocrits obtained from either diluting or concentrating whole blood using homologous plasma. Plasma and red cell recoveries are shown in Figure 15. The ruffled disk indeed reduced red cell recovery, but also reduced plasma recovery (signed-rank test: $p = 0.0313$ for both results). The greater distance between the trough and the channel reduced all channel-driven flow

\textsuperscript{11} Previous work described this as “streaking.” Although convenient, describing red cell recovery in terms of “streaking” is only qualitative. The streaking in these disks looked similar, but the hematocrit of the flowdown greatly differed.
for better or worse. Thus, placing the ruffle entrance further away from the edge of the trough has mixed effects.

Figure 15 Paired plasma and red cell recovery for disks with ruffles of channel depth 0.05 and 0.035 inch. Cutting shallower ruffles decreases both red cell and plasma recovery because the entrance to the channels is further away from the trough.

4.7 Summary

To summarize this chapter, plasma proteins from blood make the weir wettable, and ruffles induce breakthrough by bringing plasma partway into the collection area via capillary action. Ruffles have a greater effect when they cut deeply into the weir. This is an interesting phenomenon that could be used in future microfluidic designs to induce plasma flow into a collection area. If the disk design changes in the future such that the plasma must flow over a surface that had not already been exposed to the patient’s blood, then a spray-on protein coating using a common mammalian blood protein may suitably increase the surface’s hydrophilicity.

Compared to previous work, these modifications represent a modest 10% gain in plasma recovery, and a substantial decrease in red cell recovery. The best improvements to plasma
recovery were made by a combination of ruffles and wells on a glossy surface. Baffles also
induced even breakthrough but entrained more red cells. Modifications that extended close to the
trough increased red cell recovery. Future work should try to recover the meniscus fluid using
perhaps a downward-sloped weir top and wells only.
5 MODELED AND EXPERIMENTAL EFFECTS OF BLOOD DILUTION

The purpose of modeling sedimentation is to predict how the dilution of blood will change red cell and bacteria separation without having to do experiments for each proposed condition. Predicting red cell and bacteria separation purely from sensitive statistical models requires prohibitively many experiments because experimental variability is high. Instead, this section uses a numerical model to explore how red cell sedimentation changes with hematocrit. It aims to find the shortest spin time possible to remove all red cells from suspension. It does not model bacteria sedimentation, but insight from the model informs us of spin times and dilution amounts to use in carefully designed experiments in an effort to minimize red cell recovery and maximize bacteria recovery.

5.1 Sedimentation time

Previous work reduced the variables of spin time and spin speed to a single experimental variable by defining a metric called characteristic length [14]. The characteristic length is the distance that an 8-µm sphere with the same physical properties as an RBC (see Table 2 in Appendix A) would travel in a given time period when exposed to a time-varying centrifugal field. It is the time integral of Stokes velocity. Pitt et al. showed that both red cell and bacteria recovery decrease with characteristic length [14]. However, this characteristic length did not consider RBC shape, hindered settling, or the cell pack thickness.
The current model addresses these shortcomings but reduces dimensionality differently. The spatial and time domains are non-dimensionalized using parameters introduced by Lerche’s method [36], namely:

\[ r^* = \frac{r - R_{air}}{R_{wall} - R_{air}} = \frac{r - R_{air}}{L} , \quad 5-1 \]

\[ T = \frac{L}{u_{RBC,\infty}} , \quad 5-2 \]

\[ t^* = \frac{t}{T} = \frac{tu_{RBC,\infty}}{L} . \quad 5-3 \]

Here, \( r \) is the spatial coordinate measured radially outward from the air-blood interface that exists when the disk is spinning at \( R_{air} = 56.7 \) mm until the outer disk wall at \( R_{wall} = 60 \) mm. \( L \) is the nominal thickness of the fluid in the radial direction (\( L = 3.3 \) mm in the experiments of this chapter), and \( u_{RBC,\infty} = 0.638 \) mm/s is the Stokes velocity for red blood cells calculated using Lerche’s physical parameters (See Table 2 in Appendix A) evaluated at \( R_{wall} \) and at 3000 RPM. The dimensionless radial coordinate \( r^* \in [0,1] \) extends outward from the inner edge of the fluid to the disk wall. The time constant \( T = 4.7 \) s is how long a single RBC at infinite dilution would take to traverse the entire spatial domain of diluted blood, and thus \( t^* \) is the number of elapsed travel times at infinite dilution.\(^\text{12}\)

The current spin profile requires 2 minutes to decelerate from 3000 RPM in order to prevent remixing between the cell pack and the plasma, during which the Stokes velocity changes. Both the characteristic length and the conservation law models, however, assume a constant Stokes velocity. For a spin profile comprised of spin up to 3000 RPM, 0 s hold time, \( \text{12} \)

\[ \text{The conversion between } L_{\text{char}} \text{ and } t^* \text{ is } t^* = L_{\text{char}}/L. \text{ This uses the identity } L_{\text{char}} = \int_0^{t_{\text{end}}} u_{RBC,\infty}(t)dt = u_{RBC,\infty}t, \text{ where } u_{RBC,\infty} \text{ is the time-averaged Stokes velocity.} \]

51
and spin down, integrating the dynamic angular velocity profile divided by the maximum angular velocity gives the integral \( \int_0^{\text{end}} \frac{\omega(t)^2}{\omega_{\text{max}}^2} \, dt = 0.055 \). This shows that ignoring the transient parts of the spin profile only introduces a 5.5% error to the time-averaged Stokes velocity. Thus, estimates from any model that uses a constant Stokes velocity will underpredict dimensionless quantities by at most 5.5% at a hold time of 0 s. This error shrinks with increasing hold time. Furthermore, \( L \) only changes by about \( \pm 2\% \) with pipetting error of \( \pm 0.1 \text{ mL} \) (an extreme error).

The continuity equation described in Section 2.4 (Equation 2-10) is non-dimensionalized to Equation 5-5:

\[
\frac{1}{T} \frac{\partial \phi}{\partial t^*} + \frac{u_{\text{RBC,} \infty}}{L} \frac{\partial f(\phi)}{\partial r^*} = 0, \tag{5-4}
\]

\[
\frac{\partial \phi}{\partial t^*} + \frac{\partial f(\phi)}{\partial r^*} = 0, \tag{5-5}
\]

where \( f(\phi) = \phi v(\phi) = \phi(1 - \phi)hsc(\phi) \) is a dimensionless red cell volume flux determined from the hindered settling function \( hsc(\phi) \). Modeling a cylindrical system with cartesian coordinates is generally inappropriate. However, the ratio \( \frac{R_{\text{air}}}{R_{\text{wall}}} \) for the annulus-shaped fluid ring is 0.945 (5.67 cm/6 cm, see Table 5 in Appendix A), so the error is on the order of 5%. Furthermore, Detloff showed experimentally and numerically that the suspension-clear fluid (SC) interface moved at the same rate for cylindrical and cartesian geometries [49].

Numerically integrating Equation 5-5 using Godunov’s flux function (Equation 2-17) with boundary conditions \( \phi = 0 \) at \( r^* = 0 \) and \( \phi = \phi_{\text{max}} = 0.97 \) at \( r^* = 1 \) gives sedimentation profiles such as in Figure 16. Detailed code is available in Section A.6. Each plot in Figure 16 shows iso-hematocrit lines in dimensionless space and time. In each graph, the downward-sloped line crossing through \( r^* = 0 \) is the red cell suspension-clear fluid (SC) interface, and the upward-
sloping lines emanating from the origin are the sediment (or red cell pack). The steepest of these lines is the suspension-sediment (SS) interface, or the leading edge of the sediment. Both interfaces move at a constant rate until they intersect. Sedimentation time ($t_{sed}^*$) is the time required for all suspended cells to reach the sediment layer (or back wall, when applicable).

Figure 16 Red cell sedimentation profiles for a range of initial hematocrits (0.05, 0.21, 0.47). Iso-concentration lines show the dimensionless position of interfaces and hematocrit rarefaction in the sediment as a function of dimensionless time. Color supplements the iso-concentration lines and progressively gets darker with hematocrit. The suspension-clear fluid and suspension-sediment interfaces move at a constant rate until they intersect. After this, the sediment layer compacts slowly.

Figure 17 summarizes model results for dimensionless RBC sedimentation time and sediment packing ratio as a function of initial hematocrit. Hindered settling causes dimensionless sedimentation time to start at $t^* = 1$ and increase with hematocrit. Each curve illustrates a different approach for estimating sedimentation time; each differs in how (or if) it considers the sediment. The simplest ways involve finding the intersection of SC and SS interface shocks.

First, the “no sediment” model ($\nabla$) completely ignores the sediment formation. In the “no sediment” model, the suspension moves at speed $v(\phi)$ until it reaches the back wall.

Sedimentation time is simply $t_{sed}^* = \frac{1}{v(\phi)}$. This gives the longest sedimentation time and is
the least realistic. Second, the “instant compaction” model (▲) assumes that sediment does form, but that it compacts immediately to $\phi = \phi_{\text{max}}$. Here, $t^*_\text{sed}$ is the intersection in the $r^*-t^*$ plane between the SC interface

$$r^*_\text{SC} = v(\phi_0)t^*$$

and the SS interface

$$r^*_\text{SS} = 1 - \frac{f(\phi_0) - f(\phi_{\text{max}})}{\phi_0 - \phi_{\text{max}}} t^* = 1 - \frac{f(\phi_0)}{\phi_0 - \phi_{\text{max}}} t^*$$

to give the final expression

$$t^*_\text{sed} = \left(v(\phi_0) + \frac{\phi_0 v(\phi_0)}{\phi_{\text{max}} - \phi_0}\right)^{-1}.$$  

Here, rarefaction is ignored, and the SC and SS interfaces move toward each other with fixed (but different) velocity; they intersect before the suspension reaches the back wall, reducing $t^*_\text{sed}$ below that of the “no sediment” model. The “instant compaction” model is more realistic, but it does not account well for the slow compaction of red cell sediment predicted by the hindered settling corrections

The third “rarefaction” model includes the sediment and allows it to compact according to the flux curve. The “rarefaction” model is identical to the “instant compaction” model, except that the SS interface moves at a speed given by $f'(\phi_{\text{rare}})$, where $\phi_{\text{rare}}$ is the hematocrit on the sediment side of the SS interface. Although this method should be the most realistic, it gives unphysical solutions at low hematocrit in which the sediment compacts to $\phi > \phi_{\text{max}}$; the reason

---

13 The $r^*$ limits in Figure 17 makes these equations seem backward, but the equations are correct.
14 This model violates the entropy condition because the shock connecting $f(\phi_0)$ to $f(\phi_{\text{max}})$ crosses the flux curve, but it may be a useful approximation anyway because the flux curve is an empirical function.
15 $\phi_{\text{rare}}$ is the point of tangency on the flux curve connecting $\phi_0$ to $\phi_{\text{max}}$ obtained from the stretched string analogy.
is perhaps that the RBC flux curve (see Figure 1) does not give accurate values of $f'(\phi_{\text{rare}})$ for the sediment because it must extrapolate beyond the experimental hematocrits over which it was fitted ($\phi_{0,\text{exp}} \in [0.15, 0.48]$) [36]. The results of the “rarefaction” model are not shown. Lerche’s hindered settling correction (HSC) was chosen because it matched RBC compression well. The fact that the derivative of the flux curve may not accurately model compaction for a single value of $\phi$ (or $\phi_{\text{rare}}$ in the “rarefaction” model) does not invalidate the whole HSC because red cell compression occurs over all hematocrits $\phi_{\text{infl}} \leq \phi_{\text{rare}} \leq \phi \leq \phi_{\text{max}}$. Rather, the “rarefaction” model is simply unreliable for low hematocrit where the flux curve may not be well defined for the singular value $\phi_{\text{rare}}$.

Figure 17 Non-dimensional sedimentation time as a function of hematocrit. Solid markers show dimensionless RBC sedimentation time (▼) ignoring sediment formation, (▲) with instant sediment packing, and (●) from model output with rarefaction. Open circles (○) show the position of the sediment divided by the theoretical position of a completely packed sediment $\phi_0/\phi_{\text{max}}$. The vertical dashed line (---) marks the flux inflection point $\phi_{\text{infl}} = 0.348$, above which the suspension-sediment interface occurs as a rarefaction wave instead of as a shock wave.
Alternatively, the numerical solution described in Section 2.5.2 includes the sediment and allows it to compress according to the hindered settling velocity (Equation 2-6). The numerical model predicts the shortest sedimentation time: because the sediment compacts gradually, the sediment expands more for a given influx of red cells. Thus, it expands more quickly toward the suspension-clear fluid interface. Because of numerical diffusion, however, the interfaces blurred and were not strictly discontinuous. This did not affect the suspension-clear fluid shock very much, but it blurred out the suspension-sediment interface so that it propagated at speeds closer to $f'(\phi_{inflec})$ than the theoretical $f'(\phi_{rare})$. This occurred to a greater extent at hematocrits $\phi_0$ close to $\phi_{inflec}$. Improving the integration with a higher order scheme may reduce this effect. The implication of this is that the true sedimentation time may be up to 20% longer than the model suggests.

Continuing with Figure 17, the open circles show numerical model results for the ratio of sediment thickness at $t_{sed}^*$, when the red cell suspension has just barely disappeared, divided by theoretical sediment thickness ($\frac{\phi_0}{\phi_{max}}$) at complete compaction ($\phi_{max} = 0.97$). This ratio represents how “fluffed up” the sediment is relative to what it would be at complete compaction. At high and low hematocrit, the sediment that forms is already close to the maximum packing factor. Interestingly, the sediment layer is least compact before the inflection point. The reason is that as $\phi_0$ approaches $\phi_{inflec}$, the speed of the suspension-sediment interface reaches a maximum, so the sediment expands more rapidly and does not have as much time to compress before the suspension reaches it. The fact that this ratio is not constant (especially for $\phi_0 < \phi_{inflec}$) suggests that the numerical diffusion problems do not completely overwhelm the
expected physical behavior. This variable packing factor also shows that low and high hematocrits will require less time for the red cells to compact.

An obvious omission from this section is rigorous experimental validation of these various models. Lerche’s extensive validation and some brief experiments collecting sedimentation profiles of our own agreed with the numerical model results well enough that no further work was done. For example, Figure 18 shows an experimental sedimentation profile obtained by the procedure in Section A.4 overlaid with numerical model predictions for the SC and SS interfaces. The numerical model overpredicts the interface locations by 5 to 10%. This section does not boast an improvement over other models. Rather, the point of this section is to apply existing models to solve the unique problem of finding finish times.

![Figure 18 Comparison of experimental and numerical model of red cell sedimentation.](image)

Figure 18 Comparison of experimental and numerical model of red cell sedimentation. Black lines show the predicted SC and SS interfaces from the numerical model. The red shaded region is red blood cells.

Overall, results from the “no sediment”, “instant compaction” and numerical models show that predicted sedimentation time is short for dilute blood suspensions and grows quickly as
hematocrit rises. Attempts to include bacteria in these simulations have not succeeded so far, perhaps due to the complex eigenstructure of the hindered settling functions.

5.2 Bacteria recovery

Alizadeh showed that bacteria recovery decreased linearly with characteristic length ($L_{char}$, distance travelled by an 8 µm sphere) for long spin times up to $L_{char} = 250$ mm (4000 RPM for 120 s). Thus, shorter characteristic length gave better bacteria recovery [14]. Their work did not consider the effect of hematocrit on bacteria recovery. In contrast, this work focused on the effect of hematocrit on bacteria recovery for short spin times ($L_{char} < 60$ mm).

The forward flux of red cells in a centrifugal field causes backward flow of plasma. As noted in Sartory’s work in Section 2.5.1, bacteria concentration in the plasma may be higher than the initial concentration due to backward flow from the migrating red cells. Bacteria are suspended in the plasma, so if the backward plasma velocity is greater than the forward Stokes velocity of bacteria ($u_{bac,\infty} = 0.031$ mm/s), the bacteria should move backward. We hypothesize that bacteria recovery is related (perhaps linearly) to backward plasma velocity, which has the same shape as the negative of the RBC flux. Plasma backflow velocity begins at 0 for $\phi_0 = 0$ (i.e. no red cells), reaches a maximum $v_{p,abs} = -0.052$ mm/s at $\phi_0 = 0.21$ and decreases again to 0 as $\phi_0$ approaches $\phi_{max}$. Backflow velocity reaches half-maximum at $\phi_0 = 0.05$ and 0.47. Choosing hematocrits that give equal plasma velocity essentially treats backflow velocity like a factor with 2 levels. Thus, according to this backflush postulate, concentration recoveries should be equivalent at $\phi_0 = 0.05$ and 0.047 but less than concentration recovery at $\phi_0 = 0.21$. Experimental sedimentation times for these hematocrits are

---

16 Refer to Figure 23 for threshold velocity ratios to induce backflow.
approximately 4, 8, and 30 s (corresponding to characteristic lengths of 21 mm, 27 mm, and 55 mm. See Section A.4 for an example of how these times were obtained). To test this hypothesis, I created an experiment design consisting of a $3^2$ full factorial completely blocked randomized design with $n = 6$ for each treatment group. This means that blood from one donor at a time was randomly assigned to each combination of hematocrit and hold time, replicated 6 times. This is the most extensive design possible within practical experimental constraints, such as time required for counting bacteria and inter-donor blood variability.

Concentration recovery for these experiments is shown in Figure 19 (also in Table 3 of Appendix A). The overall mean concentration recovery is 0.88 (±0.05 95% confidence), which means that average bacteria concentration in the collected plasma is lower than in the saline controls. An ANOVA on concentration recovery blocked by donor (model formula: $[\text{Concentration Recovery} \mid \text{Donor} + \text{Hematocrit} \times \text{Hold time}], R^2 = 0.474$) indicated no clear effect of hematocrit ($p = 0.53, \Delta R^2 = 0.020$) or hold time ($p = 0.39, \Delta R^2 = 0.028$), but hinted at an interaction ($p = 0.09, \Delta R^2 = 0.131$). The absence of a clear hematocrit effect is evidence against the backflow theory\textsuperscript{17}. This suggests that however mobile the bacteria in the red cell suspension may be, plasma backflow does not seem to make an appreciable difference in bacterial concentration in the collected plasma.

\textsuperscript{17}Other uncontrolled sources of variation, however, may have simply masked the proposed effect of backflow on plasma concentration: the variation contained between experimental days (i.e. Donor) was the most powerful estimator of concentration recovery ($p = 0.0067, \Delta R^2 = 0.291$).
Figure 19 Concentration recovery as a function of hematocrit ($HCT_{\text{diluted}} = 0.05, 0.21, \text{ and } 0.47$) for hold times 4, 8, and 30 s. Concentration recovery does not appreciably change. 95% confidence interval shown for each group. Offset in the x-axis is for ease of display. 4 outlier replicates and 1 failed experiment are omitted from this figure and from Figure 19.

Figure 20 Bacteria recovery as a function of hematocrit ($HCT_{\text{diluted}} = 0.05, 0.21, \text{ and } 0.47$) for hold times 4, 8, and 30 s. Bacteria recovery decreases linearly with $HCT_{\text{diluted}}$ ($\beta_0 = 0.68, \beta_1 = -0.63$). 95% confidence interval shown for each group and for regression model. Offset in the x-axis is for ease of display.
Bacterial concentration is only one part of bacteria recovery: the total recovered volume also defines overall bacteria recovery. To correctly model overall bacteria recovery in our disk, we must integrate the bacteria concentration all the way from the air-plasma interface at \( r^*=0 \) to a threshold \( r_{\text{flowdown}}^* \) on the spatial axis above which everything flows down. The location of that interface in dimensionless space can be obtained by simply dividing volumetric flowdown by the total input volume to get the total volumetric recovery\(^{18}\):

\[
Volume\ recovery = r_{\text{flowdown}}^* = \frac{V_{\text{flowdown}}}{V_{\text{whole~blood}} + V_{\text{PBS}}}. \tag{5-9}
\]

In this study, the trough volumes were designed to hold all the red cells for each hematocrit, correlating volume recovery very closely with \((1 - HCT_{\text{diluted}})\). Instead of volume recovery, however, we will use \((1 - HCT_{\text{diluted}})\). Of the total suspension volume, only the fraction \((1 - HCT_{\text{diluted}})\) is collectable; so, without any enrichment of bacteria in the flowdown, bacteria recovery should decrease linearly with hematocrit. Figure 20 shows bacteria recovery as a function of \(HCT_{\text{diluted}}\). A linear model \([\text{Bacteria recovery} \mid \text{Donor} + \text{Hematocrit}]\) gives a slope \(\beta_1 = -0.633\) BR/HCT (±0.163 95% confidence, \(\Delta R^2 = 0.44\)) and intercept \(\beta_0 = 0.681\) (±0.05 95% confidence). Bacteria recovery is directly proportional to the fraction of recoverable fluid. This makes sense physically. What is surprising is that it occurs not in a 1:1 ratio. That is, dilution does not recover bacteria at as great a rate as expected. Indeed, this model predicts approximately 0 bacteria recovery at \(HCT_{\text{diluted}} = 1\), which supports the theory that bacteria recovery is proportional to recoverable fluid volume. But the fact that only 70% of the bacteria

\(^{18}\) Defining Volume Recovery in terms of “recoverable fluid” (i.e. the total input volume minus the trough volume) may be more suitable if disks are designed to hold a fixed amount of red cells, as in these experiments. Defined this way, the fraction 0.84 (±0.01 95% confidence) of the recoverable fluid was collected in these experiments, and \(r_{\text{flowdown}}^* = 0.84(1 - HCT_{\text{diluted}})/0.85\)
are recovered at $HCT = 0$ is puzzling\(^\text{19}\). At $HCT = 0.05$, fluid holdup from the trough and the residual meniscus around the weir only account for 20% of the total fluid. That means that on average, an extra 10% of the bacteria is unaccounted for. Whereas the exact cause of this inefficiency is unknown, the overall bacterial response clearly decreases with hematocrit. This suffices to answer the question of how much to dilute the blood, which is the topic of the next section.

### 5.3 Dilution optimization

When deciding how much to dilute the blood, there are several competing objectives. Diluting the blood leads to better bacterial recovery but can also drastically increase the amount of processed fluid. This section breaks down the question of dilution into a mathematical optimization problem. Downstream antibiotic susceptibility testing will likely concentrate the bacteria suspension via filtering or further centrifugation before processing it, so we care about the raw number of bacteria ($N_{bac,fd}$) that are recovered more than the concentration at which we recover them. An expression for $N_{bac,fd}$ normalized by starting concentration $C_{bac,wb}$ is:

$$\frac{N_{bac,fd}}{C_{bac,wb}} = V_{wb} f(HCT_{diluted}, t),$$

where $f = \beta_0 + \beta_1 HCT_{diluted}$ predicts bacterial recovery as a function of initial hematocrit ($HCT_{diluted}$) and hold time ($t$) from the bacteria recovery experiments. Here, $N_{bac,fd}$ increases linearly with $V_{wb}$ and decreases linearly with $HCT_{diluted}$. So, $N_{bac,fd}$ reaches a maximum at

\(^{19}\) It is true that both of these intercepts extrapolate the data. For reference, fractional bacteria recovery in pure saline is also a surprisingly low 0.61 ($\pm0.10$ 95% confidence) for a hold time of 4 seconds on the disk designed for $HCT = 0.05$. Extrapolating to $HCT = 1.0$ has no support at this time.
maximum $V_{wb}$ and minimum $HCT_{diluted}$. However, the total volume ($V_{tot}$) after to diluting whole blood has a competing trend. Solving the red cell volume balance:

$$HCT_{diluted}V_{tot} = HCT_{wb}V_{wb} \tag{5-11}$$

for $V_{tot}$ gives

$$V_{tot} = V_{wb} \frac{HCT_{wb}}{HCT_{diluted}} \tag{5-12}$$

The total volume that can be processed also increases linearly with $V_{wb}$, but inversely with $HCT_{diluted}$. We want to minimize $V_{tot}$ because it creates biological waste of which the end user (presumably a clinician) must dispose. For example, diluting blood with $HCT_{wb} = 0.55$ (the upper range of a healthy male) to $HCT_{diluted} = 0.05$ and $V_{tot} = 8.5$ mL only allows $V_{wb} = 0.8$ mL to be processed. Diluting so low reduces by nearly tenfold the amount of whole blood that can be processed. Figure 21 shows the volume of whole blood that can be processed for a range of dilutions and initial hematocrits.

Figure 21 Example curves for the bacterial recovery optimization. Number of recovered bacteria (solid line) and total processing volume (dashed line) for a range of dilution hematocrits. Each horizontal line represents 8.5mL, the current volume of fluid that is spun at a time in a 12cm disk. Displayed curves used $HCT_{wb} = 0.55$, $C_{bac,wb} = 5$ c.f.u./mL, $\beta_0 = 0.7$, $\beta_1 = -0.63$. 

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Summing Equations 5-10 and 5-12 with the positive-valued weights $w_{bac}$ and $w_{dil}$ gives the objective function

$$\min_{s.t. HCT_{diluted} > 0} V_{wb} \left[ w_{bac} (\beta_0 + \beta_1 HCT_{diluted}) + w_{dil} \frac{HCT_{wb}}{HCT_{diluted}} \right],$$

5-13

where $w_{bac}$ ($$/\text{mL whole blood}) is the perceived value of each bacterium and $w_{dil}$ ($$/\text{mL biofluid waste}) is the cost of disposing of biohazard waste. Choice of weights will be motivated by the disposal costs of processed plasma-saline solution and the benefit of recovering bacteria. The optimal dilution hematocrit is given by:

$$HCT_{diluted, optimal} = \sqrt{\frac{w_{dil} HCT_{wb}}{w_{bac} |\beta_1|}}$$

5-14

A square-root dependence on $HCT_{wb}$ in the objective function means that a lower volume of whole blood can be processed for patients with high hematocrit (such as males) for a given $HCT_{diluted}$. This objective function only considers operating costs. Capital costs will increase with dilution as the disks and the associated apparatus become larger and require more space in a clinic.

5.4 Summary

The mathematical modeling presented in this chapter shows that dimensionless spin time required to remove all red cells increases with hematocrit as the suspension moves more slowly. Dimensionless sedimentation time can be easily scaled to dimensional time using fluid volume. Experiments with blood dilution show that bacteria recovery scales linearly with the fraction of recoverable volume for the short spin times described here.
6 CONCLUSIONS AND RECOMMENDATIONS

The overall purpose of this thesis was to facilitate antibiotic susceptibility tests by rapidly separating bacteria from whole blood components which interfere with subsequent diagnostic tests. The separation process quickly removes red blood cells from a sample, leaving behind bacteria still suspended in the plasma, which is collected in preparation for antibiotic susceptibility tests. It uses a hollow centrifugal disk to leverage hydrodynamic differences between bacteria and blood components.

The specific aims of this work were to (1) maximize the fractional recovery of plasma and minimize the fractional recovery of red cells by modifying the disk, (2) explore how diluting the blood prior to spinning it might improve fractional bacteria recovery, and (3) mathematically model the separation process. This section describes the progress made toward each goal and recommends future work.

6.1 Conclusions

6.1.1 Aim 1: disk designs

There are several major findings regarding disk design that should inform future work.

First, a smooth disk surface greatly reduced variability in fractional plasma recovery. Dried plasma coatings on a smooth surface made that surface very wettable by plasma, which
encouraged plasma to flow into an area where it can be collected. Although plasma coatings also made rough surfaces more hydrophilic, the effect was not as pronounced.

Second, v-shaped channels that cut into the weir slope induced spontaneous capillary flow of plasma, which improved fractional plasma recovery relative to the base design. These channels, however, also pulled red cells into the collection area, which reduced the overall separation efficiency. These two effects from the channels grew more pronounced with increasing channel depth. Overall, three parallel v-shaped channels of half angle 20° are a helpful addition in directing plasma flow.

Third, wells, or depressions in the collection area, directed plasma flow into specific locations for collection. The disk with 8 wells and ruffles (20.05_8x3_1.25x7mm) performed the best.

Fourth, baffled disks recovered comparable amounts of plasma to disks with ruffles, but they also entrained more red cells at similar hematocrits. Future designs should exclude baffles.

6.1.2 Aim 2: blood dilution and bacteria recovery

This work characterized the effect of blood dilution on bacteria recovery and suggested an optimal dilution hematocrit as a function of costs and donor hematocrit. There are several major conclusions.

First, because of the natural variation in these experiments, we could not measure the effect of backflow on bacterial concentration. We believe on physical grounds that backflow does exist, but that its effect was not detectable given other uncontrolled experimental variables.
Second, the optimal hematocrit to which blood should be diluted scales with the square root of the donor’s whole blood hematocrit. The ultimate choice of dilution will depend on clinician operating and disposal costs.

Third, the flow of diluted blood is easier to direct, and the blood contains enough proteins to create a plasma-wettable film on the 3D printed material.

6.1.3 Aim 3: numerical model of sedimentation

Bacteria are separated from blood cells by removing the latter from suspension, and the time it takes to remove all suspended red blood cells is called sedimentation time. Several mathematical models for sedimentation time were explored, and their outcomes are described here.

First, in all four models ("no sediment", "instant compaction", "rarefaction" and the numerical model), sedimentation time started out short and increased with hematocrit. Differences between model predictions grew with different starting hematocrit. Dimensionless sedimentation time can be used to relate total processing volume with required spin time.

Second, the numerical model gives the most realistic estimates for sedimentation time because it gives full detail for sediment compaction over time. For dilute blood, red cells sediment much faster than bacteria, so the disk should only spin until all the red cells are gone, since longer times will also reduce bacterial recovery.

Third, we adopted a Riemann-solver approach to model sedimentation in multicomponent mixtures. This worked well for the 2-component (red cell and plasma), which is the numerical model presented herein. The Riemann-solver approach has not succeeded so far for a 3-
component model (red cells, bacteria, and plasma), perhaps due to the complex eigenstructure of the hindered settling functions.

### 6.2 Recommendations for future research

Future disk designs should consider the following things. Adding ruffles is beneficial on disks with wider weirs, but the width of the weir should be narrow. Thus, future disks might succeed in recovering more of the residual fluid from the meniscus by having a narrow weir and adding wells only to the collection area. Another problem with the overall disk design right now is that it requires manually collecting the plasma and transferring it to other containers for antibiotic resistance profiling. Future work may consider removing the weir and transferring the plasma directly into a downstream compartment using v-shaped channels pre-coated with PEO or plasma.

A problem introduced by diluting blood to a pre-determined hematocrit is that it takes at least 10 minutes to determine the whole blood hematocrit. The goal of this research is to minimize antibiotic detection time. Measuring donor hematocrit is a bottleneck in the process that should be shortened or eliminated. One way to do this would be to use previous blood diagnostics if available (such as for previously admitted patients) or to assume a whole blood hematocrit based on the donor’s sex. Then a single disk can be designed to hold the red cells of a sample with pre-determined diluted hematocrit and total volume.

Regarding multicomponent modeling, future efforts should investigate integration schemes that do not require full knowledge of the sedimentation equation’s characteristic structure, which becomes complex even with relatively few components. As an alternative to a full multicomponent model, future work could find a way to calculate the concentration of each
component in each suspension layer as a function of velocity ratios between each component. In whole blood, red cells are the only component concentrated enough to significantly induce hindered settling. Thus, suspension layers comprised only of slower-moving particles, such as bacteria and platelets, are diluted enough that interface locations may be calculated using the particles’ respective Stokes velocities. The location of each suspension layer, along with the concentration vector in each layer, may be used to estimate fractional recovery of each suspended component.

Another opportunity for improving modeling in future work is to consider the physical disk as a 2-compartment model comprised of the trough and the vestibule.
REFERENCES


APPENDIX A

A.1  Tables

Tables are included in this section to give pertinent physical parameters or to summarize data already presented in graphs.

Table 2  Physical properties of blood and bacteria. Lerche’s values were used in all cases except in characteristic length.

<table>
<thead>
<tr>
<th>Property</th>
<th>Pitt red cell values [14]</th>
<th>Lerche red cell values [36]</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$ (µm)</td>
<td>4</td>
<td>2.67 ± .13$^1$</td>
<td>0.84$^2$</td>
</tr>
<tr>
<td>$\rho$ (kg/m$^3$)</td>
<td>1098</td>
<td>1110 ± 1</td>
<td>1085</td>
</tr>
<tr>
<td>$\rho_{plasma}$ (kg/m$^3$)</td>
<td>1024</td>
<td>1011 ± 1</td>
<td></td>
</tr>
<tr>
<td>$\mu_{plasma}$ (kg/m s)</td>
<td>0.0012</td>
<td>0.00099 ± 0.00004</td>
<td></td>
</tr>
<tr>
<td>$u_\infty$ (m/s)</td>
<td>$13 \cdot 10^{-4}$</td>
<td>$6.38 \cdot 10^{-4}$</td>
<td>$0.31 \cdot 10^{-4}$</td>
</tr>
</tbody>
</table>

$^1$ Equivalent spherical radius for red cell of volume 95 fL.

$^2$ Equivalent spherical radius for a bacillus (rod-shaped bacteria) of volume 0.65 µm$^3$ [57].

Table 3  Recovery values for $3^2$ factorial experiments. Each row is a treatment combination. Columns show group means and standard deviations for bacteria, concentration, plasma, and red cell recovery.

<table>
<thead>
<tr>
<th>HCT</th>
<th>Hold time (s)</th>
<th>n</th>
<th>Bacteria</th>
<th>Concentration</th>
<th>Plasma</th>
<th>Red cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
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<tr>
<td>0.05</td>
<td>4</td>
<td>6</td>
<td>0.71</td>
<td>0.13</td>
<td>0.91</td>
<td>0.15</td>
</tr>
<tr>
<td>0.05</td>
<td>8</td>
<td>5</td>
<td>0.62</td>
<td>0.10</td>
<td>0.80</td>
<td>0.13</td>
</tr>
<tr>
<td>0.05</td>
<td>30</td>
<td>5</td>
<td>0.62</td>
<td>0.19</td>
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<td>0.23</td>
</tr>
<tr>
<td>0.21</td>
<td>4</td>
<td>5</td>
<td>0.55</td>
<td>0.15</td>
<td>0.89</td>
<td>0.23</td>
</tr>
<tr>
<td>0.21</td>
<td>8</td>
<td>6</td>
<td>0.56</td>
<td>0.13</td>
<td>0.89</td>
<td>0.21</td>
</tr>
<tr>
<td>0.21</td>
<td>30</td>
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<td>0.52</td>
<td>0.10</td>
<td>0.83</td>
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<td>0.47</td>
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<td>5</td>
<td>0.32</td>
<td>0.08</td>
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<td>0.47</td>
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<td>5</td>
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<td>0.10</td>
<td>0.91</td>
<td>0.20</td>
</tr>
<tr>
<td>0.47</td>
<td>30</td>
<td>6</td>
<td>0.41</td>
<td>0.06</td>
<td>1.05</td>
<td>0.18</td>
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</tbody>
</table>

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Table 4 Summary of flowdown mass, plasma recovery, and red cell recovery for all disks.

<table>
<thead>
<tr>
<th>Disk Type</th>
<th>Flowdown mass (g)</th>
<th>Plasma Recovery (%)</th>
<th>Red cell Recovery (%)</th>
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</thead>
<tbody>
<tr>
<td>Base</td>
<td>23</td>
<td>2.8</td>
<td>58.9</td>
</tr>
<tr>
<td>base</td>
<td>13</td>
<td>3.2</td>
<td>67.2</td>
</tr>
<tr>
<td>base_2mm_weir</td>
<td>10</td>
<td>2.4</td>
<td>48.1</td>
</tr>
<tr>
<td>Wells</td>
<td>28</td>
<td>3.9</td>
<td>82.2</td>
</tr>
<tr>
<td>20.05_8x3_0.75x9mm</td>
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<td>3.9</td>
<td>83.8</td>
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<td>3.9</td>
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<td>Baffles</td>
<td>27</td>
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<td>16b</td>
<td>13</td>
<td>4.2</td>
<td>85.9</td>
</tr>
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<td>matte_70.05_16b</td>
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<td>71.3</td>
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Table 5 Selected dimensions for disks. The column for fluid thickness corresponds to the variable L in chapter 5.

<table>
<thead>
<tr>
<th>Disk name</th>
<th>Weir top (mm)</th>
<th>Vestibule height (mm)</th>
<th>Trough depth (mm)</th>
<th>Trough volume (mL)</th>
<th>Total volume (mL)</th>
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</tbody>
</table>

1 48 channels on weir
2 2 channels between each baffle
3 Weir fully lined with channels
4 Thicknesses not reported for matte disks due to unknown true trough volume.
A.2 Figures

Figures in this section either provide supplemental information or summarize results for individual disks.

Figure 22 Red cell recovery versus whole blood hematocrit for individual disks. Red cell recovery increases with hematocrit.

Figure 23 Ratio of Stokes velocities to induce backflow for the second species in a 2-component suspension. Lines show the roots of Equation 2-7 solved for the absolute velocity of a second cell type using Equation 2-6. To the right of the line, the second cell type has negative velocity. Whole blood operates in the circled region.
A.3 Hematocrit calibration

Hematocrit measurements for blood samples over a range of hematocrits were collected on the lab centrifuge (Horizon Model 642E Centrifuge: spun once at 1300xg for 10 minutes) and compared to a clinical centrifuge (Thermo Scientific Heraeus Pico 17: spun twice at 10,000xg for 5 minutes). Each blood sample was split into 6 tubes and divided evenly between the lab and the clinical centrifuge. Samples in the clinical centrifuge were spun twice to complete packing. Results are shown in Figure 24. A regression on the group means ($n = 3$ for each group) gives a slope of 0.955 ($±0.003$ 95% confidence, d.f. = 7), indicating that the lab centrifuge overpredicted hematocrit by 4.5% ($±0.3$ 95% confidence). All hematocrit values reported herein were from the lab centrifuge.

![Hematocrit calibration graph](image)

$\text{HCT}_{\text{clinical}} = 0.955 \times \text{HCT}_{\text{lab}}$

$\mathbf{r}^2 = 0.999$

Figure 24 Hematocrit calibration for lab centrifuge and clinical centrifuge. Measured hematocrits for clinical centrifuge and lab centrifuge plotted against the expected hematocrit by dilution of settled whole blood. Our measured hematocrits overpredict clinical hematocrits by about 5%.
A.4 Experimental sedimentation profiles

To validate Lerche’s hindered settling correction, we recorded sedimentation profiles for hematocrits 0.05, 0.15, and 0.30 in flat cylindrical disk with a transparent polycarbonate lid. The video was converted into a stack of images spaced 1/6th of a second apart and sliced along the time axis at a constant radial position using imageJ [58]. Figure 25 shows these sedimentation profiles. The y axis cuts through the layer of blood \( L = 4.5\text{mm} \), and each column of pixels is 1/6th of a second. The suspension velocity and finish time were measured by the slope of the interface and by the end of the linear sedimentation portion, respectively. The suspension velocity agreed well with Lerche’s predictions.

![Figure 25](image)

Figure 25 Experimental sedimentation profiles in a flat, cylindrical disk for hematocrits of 0.05, 0.15, and 0.30. The x axis is the fluid plug \( (4.5\text{mm}) \). Each rows of pixels is 1/6th of a second. This disk was a flat cylinder (i.e. no trough) with a polycarbonate lid.

A.5 Effect of long hold time on plasma recovery

Alizadeh previously showed that plasma recovery decreases with characteristic length. She concluded that plasma drying made the weir less wettable, so that a lesser volume of plasma flowed down. This may have been the case on the matte disks she used, where the support material did not dry quickly. I observed linear decrease in the mass of collected flowdown 1.19
mg/s (±0.8 95% confidence, blocked by donor) using glossy disks. I posit that the fluid simply evaporates with time. I have done no other work to justify this claim.

![Graph showing fluid loss with spin time](image)

Figure 26 Fluid loss with spin time. Blood diluted to $HCT_{diluted} = 0.05$ was spun for hold times of 30, 90, and 180 s. Disk: 20.05_8x3_1.25x7mm_H5

A.6 Numerical model code

This section contains python code for the 1D, 2-component sedimentation problem discussed in Chapter 5. The module velocity_functions.py contains hindered settling corrections and is located in full at [https://github.com/cliffanderson720/MS-Thesis-Pitt](https://github.com/cliffanderson720/MS-Thesis-Pitt).

```python
1. import numpy as np
2. import velocity_functions as vf # contains hindered settling functions
3. from scipy.integrate import odeint

4. class driver:
5.     '''
6.     Sets up and solves 1D scalar sedimentation problem
7.     '''
8.     def __init__(self, H0, ngrd=100, L=1, tend=2, ntimes=20,
9.                     hsc=vf.michaels, cfl=0.95):
```
# get initial condition and hindered settling function
self.H0 = H0
self.hsc = hsc

# set spatial grid-----------------
self.ngrd = ngrd
self.L = L
self.ngrdf = self.ngrd+1
self.\Delta x = self.L/self.ngrd
self.xf = np.linspace(0,self.L,self.ngrdf)
self.x = (self.xf[1:] + self.xf[:-1])/2

## set time grid-----------------
self.tend = tend

# get max dimensionless speed
self.v0 = self.get_c(self.H0)

# adjust dt if cfl is specified
# if user has changed CFL, set ntimes using user CFL and dx
if cfl not in self.__init__.__defaults__:
    self.cfl = cfl
    self.dt = self.cfl*self.\Delta x/self.v0
    self.times = np.arange(0,self.tend+self.dt,self.dt)
    self.ntimes = len(self.times)
else:
    self.ntimes = ntimes # else, just use the default ntimes
    self.times = np.linspace(0,self.tend,self.ntimes)
    self.cfl = self.v0*(self.times[1]-self.times[0])/self.\Delta x

# Give initial concentration profile and integrate
self.\phi_0 = np.ones(ngrd)*self.H0
self.soln = abs(self.integrate())
return

def get_v(self,\phi,volavg=True):
    
    Returns velocity for a given \phi.
    volavg (Boolean) specifies absolute (True) or slip velocity (False).
    
    c = np.ones_like(\phi)
    if volavg:
        v = self.hsc(\phi)*(1-\phi)
    else:
        v = self.hsc(\phi)
    return v

def godunov(self,\phi,t,\phi_max):
    
    Godunov flux function with boundary conditions.
    Sets up d\phi/dt with entropy-satisfying fluxes in a method of lines formulation.
    Called by method "integrate"
    
    # set wall boundary conditions

82
\[
\phi_{bc} = \text{np.zeros}(\text{self.ngrd}+2)
\]
\[
\phi_{bc}[-1] = \phi_{\text{max}}
\]
\[
\phi_{bc}[1:-1] = \phi
\]

# compute flux for each cell using volume-averaged \( \phi \)

\[
q = \text{self.get_c}(\phi_{bc}) \times \phi
\]

# evaluate fluxes at each cell wall

\[
qf = \text{np.zeros}(\text{self.ngrdf})
\]

for \( i \) in range(\text{self.ngrdf}):
    if \( \phi_{bc}[i] \geq \phi_{bc}[i+1] \):
        \( qf[i] = \max(q[i], q[i+1]) \)
    elif \( \phi_{bc}[i] \leq 0 \) and \( 0 \leq \phi_{bc}[i+1] \):
        \( qf[i] = 0 \)
    else:
        \( qf[i] = \min(q[i], q[i+1]) \)

# Method of lines formulation

\[
d\phi/dt = 1/\text{self.\Delta x} \times (qf[:]-qf[1:])
\]

return \( d\phi/dt \)

---

**Lerche's hindered settling function from velocity_functions.py:**

```python
1. def michaels(a,n=2.71,amax=0.95,slip=True):
2.     """
3.     Michaels bolger correction used in Lerche’s 2001 paper.
4.     In the paper, it uses \((1-a)^2\) and is an absolute velocity.
5.     In this module, I divide by \((1-a)\) to make it a slip velocity.
6.     """
7.     if slip:
8.         return \((1-a)*(1-a/amax)^n\)
9.     elif slip==False:
10.        return \((1-a)^2*(1-a/amax)^n\)
11.    else:
12.        raise Exception('Incorrect type given for argument "slip"')
```