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A microfluidic system to study cytoadhesion of *Plasmodium falciparum* infected erythrocytes to primary brain microvascularendothelial cells

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Abstract

The cellular events leading to severe and complicated malaria in some *Plasmodium falciparum* infections are poorly understood. Additional tools are required to better understand the pathogenesis of this disease. In this technical report, we describe a microfluidic culture system and image processing algorithms that were developed to observe cytoadhesion interactions of *P. falciparum* parasitized erythrocytes rolling on primary brain microvascularendothelial cells. We isolated and cultured human primary vascular endothelial cells in a closed loop microfluidic culture system where a peristaltic pump and media reservoirs were integrated onto a microscope stage insert. We developed image processing methods to enhance contrast of rolling parasitized erythrocytes on endothelial cells and to estimate the local wall shear stress. The velocity of parasitized erythrocytes rolling on primary brain microvascularendothelial cells was then measured under physiologically relevant wall shear stresses. Finally, we deployed this method successfully at a field site in Blantyre, Malawi. The method is a promising new tool for the investigation of the pathogenesis of severe malaria.

Background

Cellular events associated with malaria mortality are highly varied and poorly understood.¹ As a malaria parasite invades and grows within an erythrocyte, it extensively alters the cell's plasma membrane by exporting proteins to the erythrocyte surface. These proteins facilitate cytoadhesion to ligands expressed by vascular endothelial cells.²⁻⁴ Autopsy results from patients with fatal malaria infections have revealed that parasitized erythrocytes often accumulate in the deep capillary beds of the brain, kidney, lungs, or intestine by cytoadhering to endothelial cells that express tissue-specific ligands.¹ As the parasitized erythrocytes accumulate in an organ's capillary network, they are thought to cause both an inflammatory response and mechanical impairment of blood flow, which can ultimately lead to organ failure.¹ Detailed characterization of parasite-endothelial cytoadhesive events that lead to organ failure is crucial to understanding the pathogenesis of complicated *P. falciparum* infections.

Directly observing cell-cell interactions within affected organs *in vivo* is generally not feasible owing to technical and ethical constraints, though imaging capillary flow in the

rectal mucosa has been performed in patients with malaria, but as impairment of blood flow in this region is not a recognized feature of severe malaria, this method can only approximate pathogenic processes in other organs.⁵ *In vitro* experimental models with varying degrees of complexity have been developed to help understand parasitized erythrocytes-endothelial cell interactions. Initially, static adhesion assays were used to observe and characterize parasite-protein and parasite-endothelial cell interactions.⁶ Introduction of parallel-plate flow chambers permitted the ability to study cytoadhesion of parasitized erythrocytes to specific surface-adsorbed proteins, transgenic mammalian cell lines, and vascular endothelial cell lines.⁷⁻¹³ Parallel-plate flow chambers offered the additional advantage of well defined fluid dynamics. Experiments using parallel-plate flow chambers revealed the phenomenon of parasitized erythrocytes rolling or 'tank-treading' over surfaces and cells displaying specific ligands. These tools have identified ligands such as ICAM-1, CD-36, and chondroitin-4-sulfate that wild type *P. falciparum* isolates have strong binding interactions and these interactions have been associated with the pathological symptoms of complicated malaria infections.^{8, 14}

In spite of these general advances, mechanistic links of how interactions of host-parasite receptors relates to malaria pathology remain weak at best. Over time, cultured parasites undergo antigenic variation in expression of their surface adhesion proteins which complicates investigation of specific parasite-ligand interactions.^{15, 16} Consequently, replicating disease pathology in the laboratory models is difficult when there is a time lag between collecting parasites and endothelial cells from patients in an endemic area and then performing experiments in a distant laboratory. An important technical challenge in understanding malaria pathogenesis is to study interactions between primary endothelial cells and fresh parasite isolates. We reported development of a microfluidic device with which we observed rolling of parasitized erythrocytes on transgenic CHO cells expressing ICAM-1 and CD36 in a laboratory setting.¹⁷ A suite of technical and conceptual changes were required to make this approach suitable for a field setting.

Microfluidic devices offer a flexible platform for performing a wide variety of experiments aimed at studying the behavior of particles and cells under flow conditions similar to those observed in the microcirculation. In particular, microfluidic devices have been utilized to study reduced deformability of *P. falciparum* parasitized erythrocytes.^{18, 19} Previous endothelial culture methods have used a technique by which adhesive cells were initially cultured on glass cover-slips and then assembled into a flow chamber.²⁰ Several groups have described microfluidic devices that have been developed specifically to study cytoadhesion events. Some early examples of these microfluidic studies have investigated interactions of platelets and erythrocytes with endothelial cells in microfluidic environments.²¹⁻²⁴ Examples of other novel devices which incorporate variable flow regimes have been utilized for studies involving platelets and particles adherent to either endothelial cells or to adsorbed protein.^{25, 26} In some cases these microfluidic devices have been utilized to culture endothelial cells for angiogenesis experiments.²⁷ Recently described microfluidic culture systems have used gravity, syringe pumps, or intermittent pumping to exchange media for cell culture. Flow through the microfluidic devices is governed by Poiseuille's Law which relates the pressure difference across the microfluidic channels to the fluid flow rate. These microfluidic systems are pumped by creating a pressure differential across a channel which then drives fluid flow through the microfluidic device from the high pressure region to the lower pressure.

In this paper, we describe a microfluidic culture system (μ F-CS) and image analysis methods developed to study cytoadhesion of malaria parasite isolates to primary human brain microvascular endothelial cells at a field site in Blantyre Malawi. The μ F-CS consisted of the microfluidic device (μ F-D), tubing, interconnect, a miniature peristaltic pump, a

stepper motor, pressure sensor, stepper motor controller, and stage mount. The μ F-D developed for this study was simple to construct with a minimum number of fluidic connections, so the devices could be manufactured in large numbers. The μ F-CS was closed loop so that connections were maintained as the device was moved from cell culture incubator to the microscope stage. The μ F-D is designed to allow reliable loading of microfluidic channels with a minimum number of endothelial cells, thus permitting multiple experiments with minimum culturing of primary endothelial cells. The system was designed to provide continuous perfusion of the microfluidic channels over several days so that endothelial cells would multiply and grow within the channels under flow. A frame averaging technique was developed to enhance contrast of slow rolling parasitized erythrocytes. The frame averaging technique was then used to augment image tracking methods available in the software MetaMorph. Image analysis techniques were developed to estimate the wall shear stress by measuring the velocity of erythrocytes moving through the field of view. Together these tools were used, at a field site in Africa, to demonstrate the feasibility of observing recently isolated parasitized erythrocytes rolling on primary brain microvascular endothelial cells under a range of biologically relevant wall shear stresses.

Materials and Methods

Endothelial Cell Isolation and Culture

Primary micro-vascular brain endothelial cells were collected from a fatal cerebral malaria infection using procedures performed in accordance with the Malawi College of Medicine Research Ethics Committee approval and approval from the Michigan State University IRB. Informed consent was obtained from the appropriate family members before autopsy. A 2 cm³ portion of frontal lobe was collected at time of autopsy and placed in 40 mL of PBS with ABAM (GIBCO BRL, Grand Island, NY). The tissue was transferred to a sterile petri dish and minced using sterile scissors and forceps. The suspension was transferred to a 15 mL conical and centrifuged at 800 G for minutes. The pellet was resuspended in 5 mL of digestion buffer (25 mM HEPES, 5 mM glucose, 120 mM NaCl, 50 mM KCl, 1 mM calcium chloride, 1.5% bovine serum albumin, and 5 mg/mL collagenase/dispase). This suspension was incubated at 37°C for 1 hour with occasional shaking. The solution was then centrifuged and the pellet re-suspended in 5 mL of endothelial cell media and passed through a 210 μ m filter. The filtrate was washed twice in PBS/ABAM and then re-suspended in 5 mL 0.05% Trypsin-EDTA (GIBCO-BRL) and incubated for 15 minutes at 37°C. The reaction was stopped by adding 5 mL of endothelial cell media, and centrifuged. The pellet was re-suspended in endothelial cell media and filtered through a 110 μ m filter. The filtrate was washed once and re-suspended in 7 mL of endothelial cell media and cultured in a T-25 vented flask at 37°C, 5% CO₂ with daily media changes.

Malaria Parasite Culture

The malaria parasite culture was isolated from a cerebral malaria patient via venipuncture and then maintained in RPMI 1640 supplemented with 2.5 wt % Albumax II and gentamicin. Malaria parasites were cultured as described previously at 2% hematocrit in O+ blood under a blood gas mixture of 2.5% O₂, 5% CO₂, and 92.5% N₂.²⁸ For this study only a single parasite isolate was used.

Microfluidic Device Fabrication

PDMS μ F-D were fabricated using single layer replica modeling. Channel patterns were designed using AutoCAD (Autodesk) based on a simplified version of c-cup shaped cellular traps published previously.²⁹ The channels between the cellular traps were 100 μ m wide and 1 mm long. These long channels are the capillary regions designed to observe parasite-endothelial cell interactions. Positive photomasks were generated (Photo Sciences Inc.) and

wafers were patterned using AZ4620 photoresist using standard protocols. The wafers were then etched in a Bosche deep reactive ion etch process (Oxford Instruments ICP 380) to produce channel depths of 30 μm . The silicon masters were vapor primed with the release agent (tridecafluoro-1,1,2,2-tetrahydrooctyl)-trichlorosilane (Gelest) in a vacuum desiccator overnight. PDMS was then mixed 1:10 monomer to hardener and poured over the wafers and cured at 140°C for 50 minutes. The channels were then released and punched using a sharpened 14 gauge blunt needle (Small Parts). Glass cover slips (Gold Seal #3334) and PDMS channels were rinsed with ethanol and then exposed to oxygen plasma of 10 watts for 40 seconds (Harrik Plasma Cleaner PDC-001). After exposure to the plasma, the cover slip and PDMS channel were brought into conformal contact to create the $\mu\text{F-D}$. Connections were made to the channels using 15 gauge 45° blunt syringe needles (Small Parts) and 1.14mm silicon auto-analysis tubing (Cole-Parmer). Stop cocks and manifolds (Cole-Parmer) were used to control flow through the device.

Pump Description

The pump system operates by maintaining a constant pressure across the $\mu\text{F-D}$. A manifold (Cole-Parmer) is configured such that the pressure sensor can measure the pressure drop across the $\mu\text{F-D}$. A microcontroller compares a voltage from the pressure sensor to a set point voltage. The microcontroller was programmed so that if there is a difference between the set point voltage and the sensor voltage, the controller moves a stepper motor (Lin Engineering) connected to a peristaltic pump head (Watson-Marlow 400A/F) to either pump fluid toward or away from the μFD . A 3mL syringe with at least a 1 mL air-bubble is placed between the peristaltic pump and the μFD . The air-bubble in the syringe acts both as a bubble trap and as a pressure dampener to reduce pressure fluctuations associated with stepper motor movement. The pump controller was designed using the Luminary Micro Stellaris® Stepper Motor Reference Design Kit (Texas Instruments) modified to accept input from an analog pressure sensor. The stepper motor microcontroller was programmed by Games4you LLC using the IAR Embedded Work bench to modify the code provided in the Stepper Motor Reference Design Kit. The pressure sensor amplifier was designed using PCB artist (Advanced Circuits) using a surface mount pressuresensor(Honneywell26PC01SMT) and an instrumentation amplifier (INA114 Texas Instruments). All components were mounted on anodized aluminum stage inserts designed for a Prior motorized stage so that they could be viewed on a Nikon TE2000-S (Nikon USA) inverted microscope. All components could be easily transferred to a cell culture incubator without disconnecting any fluid connections.

Microfluidic Endothelial Cell Culture

All channel tubing, manifolds, and media reservoirs were either sterilized with ethanol or autoclaved, and assembled as shown (Figure 1A). Endothelial cells were passaged using 0.25% trypsin-EDTA when cell cultures reached about 80% confluence in a T-25 flask. Approximately $1-4 \times 10^5$ cells were then placed in 250 μL of media and introduced into the channels by removing a connection, pulling the endothelial cell suspension into the connector and then replacing the connector. The channels were perfused initially at a pressure of 3.5kPa for 20 minutes to move the majority of cells through the channels and trap endothelial cells in the c-cups (Figure 1B). After the cells were loaded into the channels the driving pressure was reduced to 0.7 kPa to allow continuous perfusion. The later resulted in a wall shear stress of 0.5 Pa within the narrow channels between the loading areas of the channels. The endothelial cells were cultured for 2-4 days until they reached confluence (Figure 1C-1D).

Microfluidic Cytoadhesion Experiments

P. falciparum parasites were cultured as previously described.²⁸ When the parasite cultures were primarily in the trophozoite stage, cells were adjusted to 5% parasitemia and 2% hematocrit. Parasites were introduced to the device by removing one of the two upstream connectors and drawing about 200 μ L of parasite culture into the connector. After replacing the connector, flow through the device was restarted. Cell flows in channels were viewed using a Nikon TE2000-S (Nikon USA) with a 20X ELWD DIC lens. Sequential image sequences or image stacks were recorded using a Photometrics CoolSnap EZ camera. Videos were recorded using 1 ms exposure times using MetaMorph version 7.4 (Molecular Devices). Parasitized erythrocytes were identified by the presence of hemozoin and obvious contrast differences between parasitized and normal erythrocytes. Data shown are accumulated over 5 independent experiments (Figure 4B).

Frame-Averaging Contrast Enhancement

The raw image stacks were processed to facilitate data analysis on adhered cells. When necessary, frame averaging was used to enhance contrast by removing background cells and channel features so that slower moving cells were visualized in the image sequences. The slowly moving cells were usually rolling parasitized erythrocytes but occasionally uninfected cells caught on a physical obstruction (Figure 2A). Background subtraction was performed using either journal functions available in MetaMorph. Sequential images collected in the image stack were averaged to generate a frame-averaged background image. This background image was subtracted from each frame in the image stack such that the absolute value of the difference between the background and each frame was recovered. The result produced an image where moving objects appear white against a black background. The contrast for rolling and slowly moving cells was further enhanced by averaging three sequential images of this background subtracted stack. The procedure of averaging three sequential frames was repeated for each frame in the image sequence. The resulting images enhanced the contrast from any moving cell whose position overlapped the same pixels for three consecutive frames (Figure 2B). Rapidly moving cells which did not occupy the same pixels for at least two consecutive frames had their contrast reduced. These image processing techniques augmented standard methods of object tracking available through the MetaMorph object tracking plug-in (Figure 2C).

Erythrocyte Velocity Averaging

The mean velocity of erythrocytes was averaged using a routine scripted in MATLAB version 7.5.0 R2007b (MathWorks) using functions available in the Image Processing Toolbox. MetaMorph image sequence stacks had their backgrounds removed using the method described in the previous section. The images were converted to binary images by setting a threshold value. The resulting binary images were filtered such that any pixels that were present on two consecutive frames were removed. This had the effect of removing background information which was not in motion from frame to frame. Then two interrogation regions were chosen. The interrogation regions were defined as a spanning all rows of the image and enough pixel columns so the interrogation region was the same size or slightly larger than the erythrocytes imaged. The position of the initial interrogation region was chosen with *a priori* knowledge of the flow direction (Figure 3A). The second interrogation region was displaced by a certain distance in the direction of flow in the next sequential image (Figure 3B). The total number of white pixels in each interrogation region was identified. Next the difference in the number of pixels between the two interrogation regions was determined for each consecutive frame. These differences were then averaged over all consecutive frames in the movie. This process was repeated for different displacement values of the downstream interrogation window. When the difference between the two interrogation frames reached a minimum was where roughly the same numbers of

erythrocytes were in the consecutive image frames (Figure 3C). The average time between consecutive images allowed calculation of the cells mean velocity. The wall shear stress was then calculated using the depth of focus for the 20X lens which is approximately 3.6 μm . This method is insensitive to the parabolic velocity profile across width of the microfluidic channel. However, a good approximation of wall shear stresses was obtained.

Results and Discussion

Requirements and capabilities

The $\mu\text{F-CS}$ described here presents a novel solution to microfluidic studies in malaria research field settings. First, the approach provided a closed loop system that integrates all pumping onto the microscope stage. It was not necessary to disrupt fluid or pneumatic connections to transfer the culture system from the incubator to the microscope although it was possible to change electrical connections easily (Figure 1). Sterility of the channels was easily maintained during routine culture in the $\mu\text{F-CS}$. The closed loop configuration prevented contamination in the relatively dusty environment of the African field site. Incubator space in any laboratory is often limited so the ability to use multiple syringe pumps for several channels is not feasible. Second, the pumping system maintained a constant pressure across the channel so that perfusion was maintained for experiments that lasted several days. The pumping system was also integrated with the Windows-PC used to record image data so that the flow rates could be manipulated from a computer interface. This microfluidic culture system allowed easy control of flow conditions as endothelial cells grew and changed the flow environment of the channels.

Using the Microfluidic System in the Field

Growing Cells to Confluence

The $\mu\text{F-D}$ used in this paper used c-shaped cups designed to trap cells at specific points in the channels where cells were immobilized long enough to adhere to the channel walls and proliferate²⁹. These channel designs were optimized using laboratory cell lines in the US before being used to culture primary endothelial cells obtained from fatal cerebral malaria infections at the field site in Malawi. The c-shaped structures worked well for reliably loading primary endothelial cells (Figure 1B). Once the $\mu\text{F-D}$ was loaded, the endothelial cells would migrate from the traps against the fluid flow, continuing to migrate and multiply until they reached confluence after about 2-4 days (Figure 1C and 1D). The dimensions of the channels between the c-cup regions were chosen to allow the endothelial cells to migrate yet be still large enough that flow was not restricted excessively by the presence of the proliferating endothelial cells. The pumping system allowed for continuous perfusion until the cells reached confluence and beyond, until the endothelial cells had overgrown the channels, which generally occurred after 5-6 days of continuous culture.

Bubbles

Although the μFCS was a closed loop system, care was taken to avoid bubbles that would obstruct flow or compromise the endothelial cell culture. The most problematic bubbles are those that form in the tubing between the channel and the syringe, because they interfere with channel perfusion. Bubbles that form elsewhere in the fluid circuit either become trapped in the syringe or pumped into the reservoir. We found that a minimum driving pressure drop of 1.5 kPa prevented bubbles in the inlet tubing at the University of Washington in Seattle (elevation 20 m, ambient lab temperature 20-25°C). However, in Blantyre, Malawi (elevation 1000m, ambient laboratory temperature 25-30°C), only 0.7 kPa was required. Bubble formation is related to dissolved gases in the media. The lower

atmospheric pressure and higher temperature in Malawi may combine to reduce bubbles in the tubing.

Lower driving pressures, and thus lower flow rates, could be achieved by removing the reservoir. In this manner the system was completely closed and pressurized by the air bubble in the syringe, which expanded as the temperature equilibrated in the incubator to 37 C. The higher overall system pressure may have helped to reduce bubble nucleation and growth. Gas diffusion through both the PDMS and the silicon tubing appeared to be sufficient to buffer the media as the endothelial cells grew well with the μ F-CS configured in this manner.

Tracking Rolling Parasitized Erythrocytes

The design of the μ F-CS was optimized to provide a stable environment to quantify cytoadhesion of parasitized erythrocytes to endothelial cells. Uninfected erythrocytes were not observed to roll on the endothelial cells but rather occasionally bind and then release without rolling. The tracking methods offered in MetaMorph failed to reliably track rolling parasites, particularly when the parasite was obscured either by the endothelial cells or in some cases by a high concentration of erythrocytes. To overcome this problem, we averaged three consecutive frames together so that the contrast of slowly moving cells (such as rolling parasitized erythrocytes) was enhanced over that of faster moving unbound uninfected cells and the immobile background (Figure 2). This permitted the identification of rolling cells without perturbing the experiment by adding and washing away fluorescent dyes or antibody probes. Frame averaging worked well to increase contrast, but it occasionally created an effect where the rolling parasites would appear to disappear and reappear or “strobe” when they moved farther than the cell width in three consecutive frames. When this was observed, visual inspection of both the original image sequence and the frame averaged image sequence was required to properly track the cells position. Overall the frame averaging technique worked very well to augment the tracking methods available in MetaMorph.

Estimating Local Wall Shear Stress

In all μ F-D with cells, local flow can vary greatly as the cells colonize and grow within the device channels. As cells proliferate in the micro-channels, the cross-sectional area of the channel decreases, resulting in unequal flow through different sections of the device. A method to estimate the local wall shear stress was developed using erythrocytes as particles for tracking fluid velocities. Particle image velocimetry (PIV) has been performed using the erythrocytes to estimate the local flow environment.^{30, 31} PIV is measured using specialized high-speed cameras, laser light sources, and image correlation methods to describe the velocity field across the field of view. In this study, an average velocity estimation was used. The average velocity uses conventional optics and cameras and is more appropriate for field use. The average velocity method does not resolve the parabolic velocity field across the width of the micro-channel. Therefore this method is restricted in areas where flow is relatively laminar and unperturbed by obstructions. However, the method provides a useful estimate of local velocities when particles are present in at least two consecutive images across the field of view. Over the velocity range measured, the average velocity appears to be linearly related to the pressure applied across the channel (Figure 4A). This technique provides a reasonable estimate of the local flow rate through the channels, and is superior to relying on a measure of the overall pressure drop across the channel or the overall flow rate through the channel based on an infusion pump.

Rolling Velocity Versus Wall Shear Stress

A calibration plot showed that the average velocity of erythrocytes increased with the measured pressure drop across the channels (Figure 4A). The range of velocities in the

calibration plot covers the velocities observed in experiments at a constant applied pressure. Reduced flow rates with higher pressure are due to endothelial cells growing in the channels and reducing the effective cross-sectional area. Erythrocyte velocities of 5-20 mm s⁻¹ have been observed in capillaries, which translates to wall shear stresses range of 0.1 to 0.7 kPa.^{32, 33} These wall shear stresses are in the same range observed in the μ F-D described here.³⁴⁻³⁷ The rolling velocity of individual parasitized erythrocytes was observed over a variety of wall shear stresses (Figure 4B). As expected, the rolling velocity increased with the applied wall shear stress and at higher shear stresses fewer parasitized erythrocytes tended to adhere. The relatively few number of parasitized erythrocytes observed rolling on endothelial cells underscores the difficulty and unique characteristics of working with fresh parasite field isolates. Normally for similar experiments in non-endemic countries, parasite cultures are selected to enrich for expression of adhesive characteristics before binding experiments are performed. In an effort to keep the microfluidic system as close to physiologic conditions as possible, a field parasite isolate was chosen to directly demonstrate that the techniques described capture parasite-endothelial cell interactions over a variety of flow conditions. While a detailed investigation across multiple parasite isolates and primary brain endothelial cells is not presented here, the present work demonstrates that these microfluidic technologies are ready for field applications.

Conclusion

As the parasitized erythrocytes accumulate in the microcirculation it is important to understand the conditions under which they cytoadhere and how they migrate under various flow conditions. The μ F-CS described here was developed for field experimentation to observe parasitized erythrocyte cytoadhesion to primary endothelial cells. Quantifying the rolling behavior of parasitized erythrocytes over a variety of shear stresses can help describe the behavior of parasitized cells in micro-circulation. These types of measurements could help future investigations into interactions between endothelial cells and parasitized erythrocytes. This report demonstrates that microfluidic systems can be utilized to perform experiments in a malaria-endemic area. Such a system can mimic the micro-circulatory conditions in the deep capillary beds of organs and may improve our understanding of malaria pathogenesis. The μ F-CS and image analysis tools described here provide a promising new resource for investigating how cytoadhesion contributes to malaria pathology.

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Figure 1.

An image of the microfluidic culture system (I¹/4F-CS) (A). The media reservoir (1), pressure sensor/amplifier (2), stepper motor/peristaltic pump head (3), microfluidic device (I¹/4F-D) (4), and manifold (5) were all integrated onto a microscope stage insert. Only electrical connections to the stepper motor controller (6) unit need to be removed to transfer from incubator to the microscope stage. Loading cells into channels (B). Cells proliferated in the device after 48 hours (C). Endothelial cells after 72 hours formed a lawn over the entirety of the microfluidic channel while under continuous flow (D). The markings around the loading chambers are indications of chamber position on the chip. Up to four I¹/4F-CS could be maintained in an incubator for independent experiments at any given time.

Figure 2.

A typical DIC image of cells cultured in the 100 μ m wide and 30 μ m high microfluidic channels (A). Tracking parasitized erythrocytes using MetaMorph was difficult in situations where the cells were obfuscated by endothelial cells. Contrast enhancement used averaging of three consecutive frames to improve contrast of rolling cells (B) and facilitated reliable tracking of those cells (C). The advantage is this technique can improve contrast in many situations where visually tracking cells is difficult and addition of fluorescent probes is not practical.

Figure 3.

Measuring velocity of flowing erythrocytes in two consecutive frames. The background has been removed and thresholding applied to observe moving erythrocytes. The green box in (A) is held fixed while the red interrogation region in the next sequential frame (B) is displaced a certain number of pixels from the initial interrogation region. The difference in the number of white pixels between the two interrogation regions is determined. This difference is averaged for each displacement over all sequential images in the image stack (C). The minimum of this mean difference corresponds to the average distance that a cells traverse between sequential images. The average cell velocity is determined by the mean time between images and the image magnification.

Figure 4.

The mean erythrocyte velocity increased linearly with the applied pressure drop across the device (A). The wall shear stress was estimated using the erythrocyte velocity and the depth of field of the objective lens. The rolling velocity of parasitized erythrocytes (P^{M} s) increased as the estimated wall shear stress increased (B). Each dot indicates an individual Parasitized erythrocyte.

Figure 0.

We present a microfluidic culture system for studying *Plasmodium falciparum* interactions with primary brain microvascular endothelial cells.