Pf155/RESA protein influences the dynamic microcirculatory behavior of ring-stage *Plasmodium falciparum* infected red blood cells

Monica Diez-Silva1*, YongKeun Park2*, Sha Huang3, Hansen Bow3, Odile Mercereau-Puijalon4, Guillaume Deplaine4, Catherine Lavazec4, Sylvie Perrot4, Michael S. Feld5, Jongyoon Han3, Ming Dao1 & Subra Suresh1

1Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA, 2Department of Physics, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea, 3Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, MA 02139, USA, 4Unité d’Immunologie Moléculaire des Parasites, CNRS URA 2581, Département de Parasitologie/Mycologie, Institut Pasteur, 28 rue du Dr. Roux, 75734 Paris Cedex 15, France, 5G. R. Harrison Spectroscopy Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.

Proteins exported by *Plasmodium falciparum* to the red blood cell (RBC) membrane modify the structural properties of the parasitized RBC (PF-RBC). Although quasi-static single cell assays show reduced ring-stage PF-RBCs deformability, the parameters influencing their microcirculatory behavior remain unexplored. Here, we study the dynamic properties of ring-stage PF-RBCs and the role of the parasite protein Pf155/ Ring-Infected Erythrocyte Surface Antigen (RESA). Diffraction phase microscopy revealed RESA-driven decreased PF-RBCs membrane fluctuations. Microfluidic experiments showed a RESA-dependent reduction in the PF-RBCs transit velocity, which was potentiated at febrile temperature. In a microspheres filtration system, incubation at febrile temperature impaired traversal of RESA-expressing PF-RBCs. These results show that RESA influences ring-stage PF-RBCs microcirculation, an effect that is fever-enhanced. This is the first identification of a parasite factor influencing the dynamic circulation of young asexual PF-RBCs in physiologically relevant conditions, offering novel possibilities for interventions to reduce parasite survival and pathogenesis in its human host.

Shortly after invasion of the red blood cell (RBC), the malaria parasite *Plasmodium falciparum* initiates a profound remodeling of its host cell by delivering proteins to the RBC membrane. As the parasite matures, the membrane properties of *P. falciparum* infected-RBCs (PF-RBCs) are modified, with markedly decreased cell deformability and acquired cytoadherence properties. Altogether, these modifications affect the dynamic behavior of PF-RBCs altering blood flow and contributing to malaria pathophysiology.

PF-RBCs' dynamic properties are critical at the ring-stage of the infection, since this is the only asexual intra-erythrocytic stage in the peripheral circulation. Both microcapillary circuits and splenic red pulp sinuses slits dynamically challenge the mechanical properties of PF-RBCs. Several single cell assays have documented a moderate decrease in deformability of ring-stage PF-RBCs. Furthermore, there occurs a decrease in membrane flickering of PF-RBCs starting at the ring stage of the infection. Its consequences for the dynamic microcirculatory behavior of ring-stage PF-RBCs are unclear. How deformability measures of PF-RBCs assayed in static conditions translate in alteration of the microcirculatory behavior remains an open question.

In the present study, we use different approaches to document the dynamic properties and the circulatory behavior of PF-RBCs at the ring-stage of the infection and explore the contribution of the parasite-encoded Pf155/ Ring-Infected Erythrocyte Surface Antigen (RESA), which has been identified as a major contributor to the reduced ring-stage PF-RBCs deformability in static conditions. RESA is located in the dense granules of the invasive merozoites released shortly after invasion into the parasitophorous vacuole and rapidly translocated to the internal face of the PF-RBC membrane. RESA is then phosphorylated and remains associated with spectrin for the first 24 h of the asexual intra-erythrocytic development. The binding site of RESA to spectrin...
has been localized to repeat 16 of the β-chain. This interaction appears to favor the tetrameric spectrin state, resulting in membrane mechanical stabilization and increased membrane thermal stability. As a result, RESA protects Pf-RBCs from vesiculation damage induced at high temperature. Our earlier work under static conditions revealed that the role played by RESA decreasing the deformability of Pf-RBCs at the ring stage of the infection was dramatically enhanced at febrile temperature. Here we use wild-type resa1+, resa1− and resa1-rev (the revertant isogenic resa1-rev Pf-RBCs) genetically modified parasites to analyze the dynamic properties of ring-stage Pf-RBCs. The resa1-rev was constructed specifically to confirm targeted gene disruption. Since no differences in membrane stiffness between wild-type resa1+ and resa1-rev were observed, either resa1+ wild type or resa1+ revertant are used in this study as a control condition. We analyze, by diffraction phase microscopy (DPM), the membrane dynamics of ring-stage Pf-RBCs at physiological normal and febrile temperature. To assess the physiological implication of decreased deformability of ring-stage Pf-RBCs in microcirculation, we used microfluidic technology with multiple constrictions that replicate in vitro the mechanical challenges imposed to Pf-RBCs in vivo. We document in real-time the ultimate impact of RESA protein on Pf-RBCs dynamic response, quantitatively comparing transit cell velocities of individual Pf-RBCs, expressing or not expressing RESA, as they are forced to traverse successive constrictions under controlled pressure gradients. Moreover we study the effects of febrile temperature and expression of RESA on the circulatory behavior of ring-stage Pf-RBCs through a microspheres microsphiltration system, shown to mimic the mechanical challenge of Pf-RBCs by the human spleen by imitating the geometry of narrow and short inter-endothelial slits of the spleen sinuses.

**Results**

**Decreased membrane dynamics in ring-stage Pf-RBCs are RESA-driven.** We first addressed the effect of RESA on membrane dynamics of ring-stage Pf-RBCs using Diffraction Phase Microscopy (DPM). RBC samples were prepared under three different conditions: parasite-free RBCs, wild-type resa1+ and resa1-KO ring-stage Pf-RBCs (see Materials and Methods). In order to compare the dynamic membrane fluctuations of the different types of RBC samples, we calculated the root mean square (RMS) displacement of membrane fluctuation, \( \sqrt{\langle \Delta h^2 \rangle} \), which covers the entire cell area for 2 s at 120 frames/s (Figure 1A). The RMS displacement of membrane fluctuations for parasite-free RBCs was 61 ± 4.0 nm. Membrane fluctuations decreased significantly to 55.0 ± 4.3 nm in wild-type resa1+ Pf-RBCs. However, membrane fluctuations in resa1-KO Pf-RBCs showed higher values than wild-type resa1+ Pf-RBCs (62.0 ± 9.0 nm). No significant difference between membrane fluctuations in resa1-KO Pf-RBCs and parasite-free RBCs was observed.

We then retrieved the in-plane shear modulus \( \mu \), which determines the ability of RBCs to deform. The results for the shear modulus are shown in Figure 1B. For parasite-free RBCs, \( \mu = 6.2 ± 1.4 \mu \text{N/m} \). A significant increase in \( \mu \) was observed for wild-type resa1+ Pf-RBCs (15.0 ± 3.8 \mu \text{N/m}), indicating a decrease in cell deformability. Interestingly a significant decrease in \( \mu \) is observed for resa1-KO Pf-RBCs (7.6 ± 2.6 \mu \text{N/m}). These results are consistent with literature values of membrane shear modulus obtained using independent techniques.

**Figure 1** | Membrane dynamics of ring-stage Pf-RBCs at physiological body temperature. (A) RMS displacements, and (B) in-plane shear modulus values of parasite-free RBC, wild-type resa1+ and resa1-KO ring-stage Pf-RBCs. Open circles are experimental values and represent individual cell measurements. Significant differences are shown as * \(( p < 10^{-4})\) and ** \(( p < 10^{-5})\) values.

**Figure 2** | Membrane fluctuations and in-plane shear modulus of ring-stage Pf-RBCs at body and febrile temperature. RMS displacement histogram and in-plane shear modulus values (upper and lower panel, respectively) of (A), (D), parasite-free RBCs, (B), (E), resa1+, and (C), (F), resa1-KO ring-stage Pf-RBCs, measured at 37°C and 41°C. Open circles represent individual RBC measurements. Closed circles are shear modulus results obtained with optical tweezers and the same set of parasites shown for comparison purposes. Significant differences are shown as * \(( p < 0.02)\) values.
experimental methods under static conditions that involved optical tweezers tests on parasite-free RBCs and wild-type and resa1-KO ring-stage Pf-RBCs, and previous work on 3D7 ring-stage wild-type Pf-RBCs using DPM measurements.

Temperature enhances membrane dynamics of ring-stage Pf-RBCs. Membrane fluctuation values derived from the DPM measurements substantially increased from normal physiological to febrile temperature in parasite-free RBCs (Figure 2A), while the in-plane shear modulus values decreased (Figure 2D). In contrast, membrane fluctuations of resa1+ and resa1-KO Pf-RBCs were essentially similar at 37°C and 41°C (Figure 2B), but the in-plane shear modulus showed a significant decrease in cell deformability from body to febrile temperature, although the scatter range was large (Figure 2E). Resa1-KO Pf-RBCs DPM measurements showed that membrane fluctuations values were somewhat higher at 41°C than those observed at 37°C (Figure 2C), although the increase was modest compared to parasite-free RBCs. The resa1-KO Pf-RBCs in-plane shear modulus showed no significant difference between normal and febrile temperature as observed for parasite-free RBCs (Figure 2F). Results on membrane shear modulus measurements obtained previously using optical tweezers test on parasite-free RBCs, wild-type resa1+ and resa1-KO ring-stage Pf-RBCs are shown for comparison (closed circles Figure 2 D–F).

Microcirculation of ring-stage Pf-RBCs is influenced by RESA. The effect of RESA expression on the dynamic properties of ring-stage Pf-RBCs was measured using a microfluidic assay quantifying the transit cell velocity of Pf-RBCs moving through channels with multiple successive constrictions under controlled pressure, and thereby simulating host microcirculation in vitro. Figure 3 shows in real time a representative example of the dynamic response of parasite-free RBCs, resa1+ and resa1-KO Pf-RBCs when they were forced to pass through multiple constrictions at normal physiological (37°C) temperature. At a constant gradient pressure of 0.24 Pa mm⁻¹ ring-stage Pf-RBCs were able to deform and squeeze through micro-sized constrictions of 3 μm. However, when compared to the transit cell velocity of parasite-free RBCs

Figure 3 | Differences in the dynamic response of ring-stage Pf-RBCs expressing or not RESA. Real-time snap-shots transit cell velocity of a representative example of all the experiments performed of (A) resa1+, and (B) resa1-KO ring-stage Pf-RBCs, forced to traverse through 3 μm successive constrictions in micro-sized channels at a constant pressure gradient of 0.24 Pa μm⁻¹. Pf-RBCs are labelled fluorescently using Thiazole orange. Yellow arrows indicate individual parasite-free RBC. (C). Illustrative example of all the transit cell velocity measurements performed. Each open circle represents the spatial position (μm) as a function of time (s) of individual resa1+ (red) and resa1-KO (green) Pf-RBCs compared to parasite-free RBCs (grey) calculated from Figure 3A and B.

Figure 4 | Transit cell velocity of ring-stage Pf-RBCs at body temperature. Transit cell velocities of (A) resa1+, (B) resa1-KO and (C) resa1-rev ring-stage Pf-RBCs compared to corresponding co-cultured parasite-free RBCs, passing through 3 μm constrictions in micro-sized channels. Measurements were performed at 37°C at a constant pressure gradient of 0.24 Pa μm⁻¹. Each open circle represents an individual cell measurement. Significant differences are shown as * (p < 10⁻⁹) values.
were incubated at 40°C for 18–21 h, and 0.641 for 21–24 h.

were incubated at 37°C and 41°C. Measurements were performed at a constant pressure gradient of 0.24 Pa μm⁻¹. Each open circle represents an individual cell measurement. Significant differences are shown as * (p < 0.01) values.

appearing in each field of view, the transit cell velocity of individual resa1+ Pf-RBCs (Figure 3A and supplementary material Video S1) was significantly slower than resa1-KO ring-stage Pf-RBCs (Figure 3B and supplementary material Video S2). As an illustrative example of all the cell velocity measurements performed, the spatial position as a function of time of individual resa1+ and resa1-KO Pf-RBCs compared to parasite-free RBCs (calculated from Figure 3A and B) are represented in Figure 3C. The average transit cell velocity of resa1+ Pf-RBCs (14.7 μm/s) was slower than parasite-free RBCs from the same culture (26.8 μm/s, p < 0.001) (Figure 4A). However, resa1-KO Pf-RBCs and bystander parasite-free RBCs exhibited similar transit cell velocities (24.5 and 25.9 μm/s, respectively, with p = 0.186) (Figure 4B).

To further substantiate that the observed difference in transit cell velocity between resa1-KO Pf-RBCs and resa1+ Pf-RBCs was specifically due to the absence of RESA protein expression, we tested the dynamic response of the revertant isogenic resa1-rev Pf-RBCs. The resa1-rev Pf-RBCs displayed markedly decreased average transit cell velocity (16.8 μm/s) in the same range as the original resa1+ parental line, and substantially lower than bystander parasite-free RBCs (28.1 μm/s) (p < 0.001) (Figure 4C).

The effect of RESA on Pf-RBCs microcirculation in vitro was also investigated at febrile temperature. Indeed, the transit cell velocity of wild-type resa1+ Pf-RBCs was reduced (Figure 5A) when microfluidics experiments were performed at 41°C (10.2 μm/s compared to 14.7 μm/s at 37°C, p < 0.01). Resa1-rev Pf-RBCs behaved similarly, with an average transit cell velocity of 13.2 μm/s at 41°C and 16.8 μm/s at 37°C (p < 0.01) (Figure 5C). However resa1-KO Pf-RBCs, which had similar transit cell velocity values at both 37°C and 41°C (24.5 μm/s and 22.6 μm/s, respectively, p=0.1863) displayed a similar trend as the parasite-free RBCs from 37°C to 41°C (26.8 μm/s and 23.30 μm/s p < 0.01) (Figure 5B).

**Temperature and RESA expression influence filterability of ring-stage Pf-RBC in microspheres.** The effect of RESA on the circulatory behavior of ring-stage Pf-RBCs was assessed using microspheres, a microspheres filtration system simulating the narrow and short geometry of inter-endothelial slits of the spleen sinuses. Highly synchronized cultures (ring stage at 15-18 h) pre-incubated for 3 h at 37°C or 40°C were loaded on the calibrated microspheres columns. Transposal of Pf-RBCs was quantified by calculating the retention rate in the microspheres. Retention of resa1-KO and resa1-rev Pf-RBCs did not differ significantly in the culture maintained at 37°C (Figure 6A). However, it was increased by 30-60% in resa1-rev Pf-RBCs pre-incubated for 3 h at 40°C and not in resa1-KO Pf-RBCs (Figure 6A). To rule out that increased retention of resa1-rev parasites was due to a more advanced parasite developmental stage, possibly more rapid development during the 3 h exposure at 40°C, the duration of the temperature shift was reduced to 2 h and synchronous parasites at different stage of development were used. Temperature-shifted 12–15 h old or 15–18 h old resa1-rev ring stage Pf-RBCs displayed a higher retention rate compared to the sibling culture maintained at 37°C. Such a temperature-induced reduced filterability was not observed for the
**Discussion**

The results presented here demonstrate that expression of RESA protein in the ring stage of intra-erythrocytic development of *P. falciparum* has a significant effect on dynamic biophysical properties and microcirculatory response of *Pf-RBCs*. They also provide clear evidence that ring-stage *Pf-RBCs* markedly differ in their microcirculatory behavior from bystander parasite-free RBCs and from ring-stage *Pf-RBCs* devoid of RESA. Febrile temperature exacerbates the role of RESA in influencing the dynamic microcirculatory properties of *Pf-RBCs*.

Nanoscale fluctuations (commonly referred to as “flickering”) of the cell membrane at 37°C are markedly decreased by RESA expression in the ring stage. This is consistent with a reported reduction in thermally driven membrane flickering over the full range of (wild-type) infected stages of *Pf-RBCs*. It had been speculated that parasite modifications to the host RBC cytoskeleton are responsible for changes in the *Pf-RBCs* membrane flickering profile. The membrane fluctuation values at 37°C on *resa1-KO* *Pf-RBCs* and parasite-free RBCs were comparable, indicating that the presence of RESA on host membrane, or downstream effects of its expression, are involved in the membrane flickering changes observed in young stages at normal body temperature. The in-plane shear modulus data retrieved from the measured membrane fluctuations values indicate increased rigidity for the *Pf-RBCs* expressing RESA but not for *resa1-KO* *Pf-RBCs*. These results are in agreement with optical tweezers measurements of quasi-static deformation, and confirm that RESA protein modulates cell deformability of ring-stage *Pf-RBCs*.

We found febrile temperature to significantly influence the membrane dynamic properties of parasite-free RBCs present in the same culture as ring-stage *Pf-RBCs*. The membrane fluctuation of parasite-free RBCs increased from normal physiological (37°C) to febrile (41°C) temperature while the in-plane shear modulus decreased. This latter observation is in line with the reported decrease by about 20% of the shear modulus of healthy RBC membrane when the temperature increased from 23 to 41°C. This indicates an increase in the overall parasite-free RBCs deformability at febrile temperature, possibly reflecting structural changes of the RBC membrane phospholipid and/or spectrin network that alter its elastic properties. One possible contributor is the transitional structural change in α- and β-spectrin molecules near 40°C. Interestingly, different observations were made with ring-stage *resa1+ Pf-RBCs*. Since the in-plane shear modulus of RBCs in our results is calculated from the tangential component of displacement in membrane fluctuations, both the measured axial membrane fluctuation and morphology of RBCs determined shear modulus value. No significant changes in membrane fluctuations were observed upon temperature shift in *resa1+ Pf-RBCs* while the in-plane shear modulus values increased, i.e. both parameters showed opposite trends compared to bystander parasite-free RBCs. Since RESA interaction with spectrin stabilizes spectrin’s tetrameric state *in vitro*, it probably prevents the dissociation of spectrin tetramers at 41°C, resulting in overall decreased *Pf-RBCs* deformability and increased membrane mechanical stability.

This inference is also consistent with the earlier finding that RESA prevents membrane vesiculation at 50°C. The RESA-dependent temperature enhancement of ring-stage *Pf-RBCs* stiffness observed here is consistent with previous static deformability measurements obtained using optical tweezers. At 41°C, a modest increase in membrane fluctuations was recorded in *resa1-KO* *Pf-RBCs* compared to that observed at 37°C. This indicates that in addition to RESA expression, possibly other parasite-associated factors contribute to modulating ring-stage *Pf-RBCs* membrane fluctuations at 41°C. Possible parasite factors influencing membrane fluctuations changes at 41°C are additional parasite-encoded proteins and/or apical organelle-associated proteins discharged in the erythrocyte-membrane during invasion. Alternative RESA-independent modifications are proteolysis of band3 and/or of erythrocyte cytoskeletal proteins needed to ensure invasion. Although *Pf-RBCs* lacking RESA showed increased membrane fluctuations values at 41°C, the in-plane shear modulus remained essentially unaffected at 37°C and 41°C. The data confirm RESA as the main molecule involved in *Pf-RBCs* deformability changes observed at elevated temperature, and support the role played by the specific RESA-spectrin interaction at 41°C.

Analysis of the dynamic properties of *Pf-RBCs* using a microfluidic device that replicates successive constriction obstacles encountered in the vascular microcirculation was done at physiological and febrile temperatures. Transit cell velocity of ring-stage *resa1+ Pf-RBCs* was markedly decreased compared to bystander parasite-free RBCs, and was further reduced at febrile temperature. Although *resa1-KO* *Pf-RBCs* travelled slightly slower than the co-cultured parasite-free RBCs, this was not statistically significant, and moreover the transit cell velocity was independent of the temperature at which the experiments were performed. The similarly higher transit cell velocities of *resa1-KO* *Pf-RBCs* and parasite-free RBCs indicate that RESA is largely responsible for the reduced transit cell velocity of ring-stage *Pf-RBCs*. This data identifies for the first time RESA as a parasite factor able to modulate the dynamic response of ring-stage *Pf-RBCs* during microcirculation.

In addition to vascular microcirculation, another anatomical site where RBC dynamic properties are stringently challenged is the red pulp of the human spleen where the RBCs must undergo extensive deformation as they cross the narrow inter-endothelial slits of the sinuses. In an *ex vivo* organ perfusion system, a significant proportion of ring-stage *Pf-RBCs* was retained in the red pulp of the human spleen, most probably due to their altered mechanical properties. The bead microsphiltration system we used here mimics the mechanical sensing of RBCs by the human spleen, as it was shown to retain ring-stage *Pf-RBCs* at a rate similar to that observed in an isolated-perfused human spleen. This system does not permit assessment of the behavior of bystander parasite-free RBCs, but allows us to monitor the filterability of *Pf-RBCs* and hence the impact of the presence of RESA and of a temperature shift on efficiency of traversal of the microsphere layer. Filterability of *resa1-rev* and *resa1-KO* *Pf-RBCs* was similar at 37°C, but a transient incubation at 40°C resulted in increased retention of the RESA expressing *Pf-RBCs* and not of the *resa1-KO* *Pf-RBCs*. Such a temperature-associated decreased filterability of RESA-expressing parasites was observed for late ring-stage *Pf-RBCs*, and was not observed in mature stages, which no longer express RESA. The late ring stage parasites studied here were harvested before surface expression of the *P. falciparum* erythrocyte membrane protein 1 (EMP1). Furthermore no difference in retention with the bead system could be observed over the first 20 hours post invasion between K+ and K− parasites excluding any possible effect of knobs in bead retention. Thus, the microsphiltration results show that RESA expression clearly modulates the circulatory behavior of *Pf-RBCs* at febrile temperature.

Our data present the first detailed evidence for a major role of temperature and presence of RESA in modulating the dynamic microcirculation characteristics of ring-stage *Pf-RBCs*. This conclusion, stemming from the analysis of dynamic behavior of *Pf-RBCs* using complementary approaches that impose distinct geometric constraints on dynamic cell deformability, is consistent with observations of the static behavior of ring-stage *Pf-RBCs*, using optical tweezers and biophysical measurements. The consistency of data obtained on the static and dynamic behavior on the one hand and biophysical measurements on the other is interesting as it cross-validates the...
various, complementary approaches. Moreover, data presented here as well as previous studies3,5–7 point to RESA as a major determinant of the temperature-enhanced deformability defect of ring-stage Pf-RBCs.

RESA may play a bifunctional role on influencing microcirculation of ring-stage Pf-RBCs during an in vivo infection. While its expression is essential for the young ring stage parasites to resist a transient exposure to febrile temperature and ensure their normal intra-erythrocytic cycle and parasite development, exposure of febrile temperatures can possibly lead to a splenic retention and accelerated parasite clearance.

The present findings open new avenues for intervention to reduce parasite survival and pathogenesis in its human host.

Methods

Parasites and culture. The P. falciparum EPU/CB line (referred to as wild-type resolute+) and its derived resolute strain (resolute KO) and resolute-revertant clone (resolute-revert) were cultured in leukocyte-free human RBCs (Research Blood Components, Brighton, MA) under an atmosphere of 5% O₂, 5% CO₂, and 90% N₂ at 37°C in RBC culture medium (Invitrogen, Carlsbad, CA) and then added to the suspension and samples were incubated at room temperature for 20 min in the dark. Then, a 10 μL RBC suspension was flowed through the microfluidic device previously coated with PBS-1% BSA, at a constant pressure gradient of 0.24 Pa μm⁻¹. All experiments were imaged using a bright-field microscope (Olympus IX71, Center Valley, PA) equipped with a 60x objective and connected to a CCD camera (Hamamatsu Photonics, C4742-80-12AG, Japan). Images of individual RBC were acquired automatically using IPLab (Spectralys, Rockville, MD) at 70 ms time interval using a green illumination (545 nm). Post-imaging analysis was done using imageJ software (National Institutes of Health).

The temperature was controlled using a heating chamber (Olympus, Center Valley, PA), which was preheated to 30 min before the beginning of the experiment. Then a PBS-1%BSA coated microfluidic device was placed into the heating chamber 5 min before loading the RBCs suspension. A thermal sensor was used to probe the exact temperature inside the heating chamber and 5 min were required for the temperature to be adjusted to a different value.

Transit cell velocities of individual RBCs and Pf-RBCs passing through micro-channels were analyzed. Microfluidic measurements were performed at 37°C and 41°C, on ring-stage parasites (13–17 h post invasion).

Microphilltration. Microphilltration was performed using methods previously established Briefly, on physiologic red cell deformability.

Microfluidic measurements. RBCs and Pf-RBCs were centrifuged at 300 g and diluted in Phosphate buffered saline (PBS), 1% Bovine Serum Albumin (BSA), and diluted in Phosphate buffered saline (PBS), 1% Bovine Serum Albumin (BSA) and diluted in Phosphate buffered saline (PBS), 1% Bovine Serum Albumin (BSA).

Briefly, the microfluidic device consists of multiple parallel capillary channels with triangular pillars arrays, producing successive micro-sized constrictions of 3.0 μm between pillars, with a depth of 4.2 μm. The inlet and the outlet reservoir have the same dimensions of 500 x 500 μm.

![Image](image.com/scientificreports)
The red blood cell (RBC) membrane is a complex biophysical structure that plays a crucial role in the malaria parasite invasion of the human erythrocyte. The malaria parasite, Plasmodium falciparum, uses specific proteins to bind with host erythrocyte and AMA1 during merozoite invasion. The PfEMP1 protein, associated with spectrin in the erythrocyte membrane, is involved in erythrocyte invasion. The PfEMP1 protein influences the dynamic mechanical properties of the RBC membrane, which is involved in the process of invasion. The PfEMP1 protein also impacts erythrocyte mechanical properties. The PfEMP1 protein also impacts erythrocyte mechanical properties, which is involved in the process of invasion. The PfEMP1 protein also impacts erythrocyte mechanical properties, which is involved in the process of invasion.