mPharesis: continuous high gradient magnetophoretic separator for malaria-infected red blood cells

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ABSTRACT

The *Plasmodium falciparum* malaria parasite causes nearly one million deaths per year across over 100 countries. The parasite invades the host's red blood cells (RBC), feeding off of the RBC's hemoglobin and creates a magnetic byproduct within the infected RBCs (iRBC). **Even a low concentration of iRBCs often leads to death in less than 24 hours.** Therapies include parental quinine or artesunate treatments. However, parasites have become resistant to these often pricey drugs thus limiting their effectiveness. Exchange transfusion (ET) has been used as an adjunct treatment yet its efficacy remains the subject of clinical debate. **mPharesis, a magnetic dialysis-like device, is in development to remove a patient's iRBCs without removing the healthy RBCs while minimizing plasma loss.** The device, used in adjunct with drugs, provides a useful alternative to ET while being more accessible to low-resource settings where blood supply is limited. Here, preliminary data on a device prototype is reported.

The device is made in-lab with inexpensive rapid manufacturing techniques. The **strong magnetophoretic force is generated with an external permanent magnet array**. Experiments were conducted in-vitro using iRBCs and a non-pathogenic blood analog composed of a mixture of healthy and methemoglobin RBCs (metRBC) which has similar paramagnetic properties as iRBCs. Tests were conducted with multiple hematocrits and designs.

The concentration of metRBC was reduced by as much as 24.7% for 15%Hct and 14.6% for 30%Hct in a single pass at a flow rate of 0.077mL/min. Ongoing progress includes design modifications to allow for automation and increased throughput. In addition to malaria treatment applications, the mPharesis device could potentially be used as an alternative to ET with other disease management, such as Sickle-cell disease.

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INTRODUCTION

Plasmodium falciparum malaria is a mosquito-borne infectious disease causing over **660,000 deaths a year[1].**

- Lifecycle: parasite infects the host, invades circulating red blood cells (RBC), and turns RBC's hemoglobin (Hb) into **paramagnetic hemozoin crystal[2]**
- 5-60% of the host's RBCs can be infected, **mortality rates of 15-22% even with optimal treatments[3]**

Treatments are severely limited.

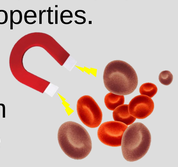
- Quinine- and artemisinin-based drug therapies often ineffective due to innate patient or malarial drug resistance. Cardiotoxicity from overdosing common
- *Exchange Transfusion* flushes patient with large volumes of donor blood (2-10L). **Blood is scarce in developing countries and carries a high transfection risk.**

High gradient magnetic separator (HGMS) – novel treatment

- Use single external magnets or magnet arrays combined with ferromagnetic bead packed columns, embedded ferromagnetic wires, and/or saline buffer clearance streams.
- Some perform well, few can operate continuously and **all of these have a very low cell throughput**.
- None HGMS can be used for treatment.

PRINCIPLE OF OPERATION

***mPharesis* (magnetic apheresis):** dialysis-like procedure to be used in conjunction with parental therapies to separate out infected RBCs (iRBCs) by capitalizing on their unique magnetic properties.



Principal of operation **(see Figure 1)**

- Infected blood flows through a 100µm tall channel with a ferromagnetic wire array (*grid*) at the bottom
- Permanent magnet lies directly below the grid plate, creating a high uniform magnetic field which attracts passing iRBCs
- **Infected blood (iRBC-enriched basement layer) is then skimmed of by thin exit *waste* slit, discarded**
- Remaining purified blood is returned to the patient

Methemoglobin RBCs (metRBC): non-pathogenic magnetic blood analog to iRBC

- Easily prepared on the bench top, stable for weeks
- Have similar magnetic susceptibility and rigidity as iRBCs[2,4].

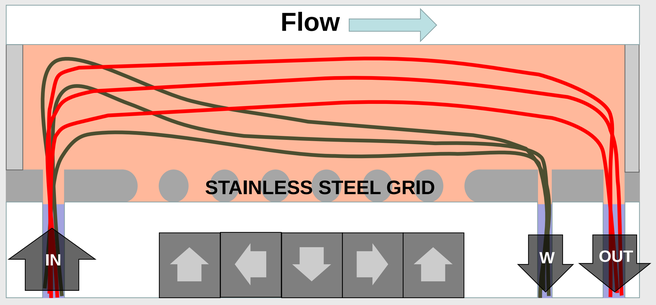


Figure 1. Simplified representation of the mPharesis principle of operation. Bright red lines represent healthy RBCs' flow path and brown lines indicate iRBCs.

RESULTS

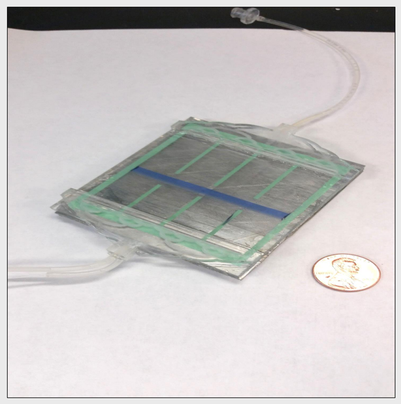
Experimental variables: 15% and 30%Hct, 0.075mL/min at 10% split ratio (Q_{waste}/Q_{inlet}). Each experiment included a control device (no magnet) and magnet device simultaneously.

The *clearance efficiency of metRBCs* ($C_{metRBCs}$) is the percent change in metRBCs from the inlet to the outlet, or what would be returned to a patient. . It is calculated using the following equation where *x* represents healthy RBC or metRBC and concentration is given by %.

$$C_x = \left[1 - \frac{Hct_{out} * \%_{x,out}}{Hct_{in} * \%_{x,in}}\right] * 100\%$$

metRBC preparation results (n=18)

- Hemoximeter analysis: 34.9±0.6%Hct, 0.2±0.0% HbO_2, 1.9±0.1% HbCO, 98.1±0.3% metHb, and 0.8±0.3% deoxygenated Hb
- Optical Shearing System: both healthy RBCs and metRBCs did not deform at 100s^{-1}, **healthy RBCs fully deformed at 500s^{-1} while metRBCs only partially deformed**



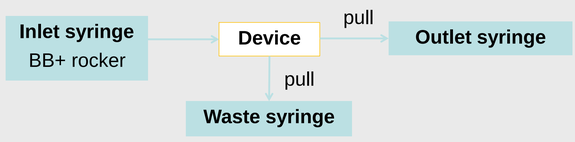


Figure 2. Top) single layer mPharesis prototype. Bottom) diagram of experimental setup

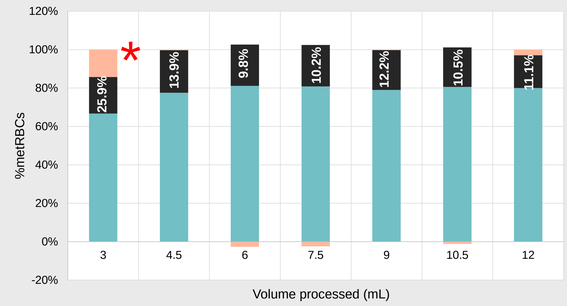


Figure 3. Saturation study results for metRBCs at 30%Hct, 20% parasitemia,10%SR, and 0.075mL/min (v=0.3mm/s) . Blue is metRBCs in outlet, black is waste. and pink is what remained in the device. White percentages represents $C_{metRBCs}$.

DISCUSSION

GOALS

- **No separation in control (no magnet)**
- **0% < C_{hRBC} < SR, ideal = 0% (no healthy in waste)**
- **SR < C_{metRBC} < 100%, ideal = 100% (no metRBC in outlet)**

Figure 3 plots %metRBCs in waste and outlet; includes $C_{metRBCs}$ in white text for saturation study:

- Avg,$C_{metRBCs}$ = 18.0%±5.2% (n=18)
- Avg,C_{hRBCs} = -1.3%±2.9% (n=18), little hRBC loss
- 10% SR and v=0.3mm/s found to be best for optimal efficiency and throughput
- Conditions: 0.075mL/min, 30%Hct, 20% metRBCs, 10% SR.
- For 30%Hct and 20% parasitemia, reaches steady state >~4mL (*).

Ongoing device developments – scaling up

- **Target treatment similar to dialysis or hemofiltration**: approx. 3-4hr session at 500mL/min, reduce the patient's parasite density by approximately 30 fold
- Multi-scale clearance models for potential treatment
- Optimized multi-device network for maximized iRBC clearance
- **Continued optimization using cheap, in-lab fabrication**

Malaria verification tests are still required, challenging!

- metRBC vs iRBC verification needed, protocol may need to be improved upon depending on results
- Future plans: obtain *P. falciparum* cultures with desired physiological parameters, at least 10mL of 15%Hct with 10% parasite density

Other possible applications of mPharesis

- Non-diluting magnetic separator using specific-binding microparticles
- Hemofiltration of damaged RBCs (e.g. dialysis or Sickle-cell patients)
- Verification device for other modified-RBC protocols

METHODS AND MATERIALS

MetRBCs were prepared using fresh whole blood from a donor obtained via venipuncture with an approved protocol. The RBCs were washed three times, the buffy coat and plasma removed, and re-suspended with DPBS. Solid $NaNO_2$ (0.069g per 1mL blood) and PBS (10mL per 1mL blood) were vortexed together then added to the washed blood. The mixture incubated in a closed, rocked container at room temperature for 90min, and then washed three times. MetRBC concentration verification was performed using a hemoximeter (OSM-3, Radiometer, Brønshøj, Denmark) to measure levels of metHb, HbO_2, and HbCO. The rigidity of the metRBC product was estimated by visual comparison of their deformation under controlled shear stress of 100 and 500s^{-1} via a Linkam Optical Shearing System.

The current mPharesis grid was photo-etched from a 125µm thick sheet of SS410 stainless steel. The acrylic grid plate, top plate, and flow splitters were laser cut and engraved with a 40W laser cutter. Acrylic layers were solvent bonded together with dichloromethane to create inlet and outlet flow path "splitters". The grid was mounted to the acrylic grid plate with double-sided 3M 444 pressure sensitive adhesive tape. The long pockets in between the poles were filled with Loctite 290 Threadlocker and 7469 Primer and then allowed to cure at room temperature with a thin polytetrafluoroethylene sheet smoothed over the surface. The grid surface was carefully planed flat with a chisel-shaped knife blade. The plenum gasket is 80µm thick polyethylene shim stock cut with a Silhouette Cameo electronic cutting machine (Silhouette America, Lehi, Utah, USA). The layers were aligned and sandwiched, then the device edges were sealed with Loctite 430 cyanoacrylate and reinforced with overlapping thin strips of acrylic.

Figure 2 shows the experimental setup. For each grid layer, 5mL of the blood mixture made of approximately 80:20 washed RBCs to metRBCs was processed through the device. Inlet syringe was continuously rocked with an included stainless steel bearing. After the experiment, the final inlet, waste, and outlet were sampled. The samples' Hct and blood content (i.e. metHb % and HbO2 %) were analyzed in the hemoximeter.

ACKNOWLEDGEMENTS

NIH 15R43HL110508-01A1, 3R01JL089456 and R01 HL089456-A1

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