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

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**INTRODUCTION**

*Plasmodium falciparum* malaria is a mosquito-borne infectious disease causing over 600,000 deaths a year. Lifestyle: parasite infects the host, invades circulating red blood cells (RBC), and turns RBC’s hemoglobin (Hb) into paramagnetic hemoglobin (HbO2 and methemoglobin [metHb]).

- 5-60% of the host’s RBCs can be infected, mortality rates of 15-22% even with optimal treatments

Treatments are severely limited.

- Quinine- and artemisinin-based drug therapies often ineffective due to innate patient or malarial drug resistance. Cardioxotoxicity from overdosing common
- Exchange Transfusion (ET) flushes patient with large volumes of fresh blood (2-10L). Blood is scarce in developing countries and carries a high transfusion 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 (metRBCs): non-pathogenic magnetic analog to iRBC

- Easily prepared on the bench top, stable for weeks
- Have similar magnetic susceptibility and rigidity as iRBCs

**METHODS AND MATERIALS**

metRBCs were prepared using fresh whole blood from a donor obtained via venipuncture with an approved protocol. The RBCs were washed and centrifuged, then added to the washed blood. The mixture was incubated in a closed, rocked container at room temperature for 90min, and then washed three times. Methemoglobin RBC concentration verification was performed using a hemoximeter (OSM-3, Radiometer, Brønshøj, Denmark) to measure levels of metHb, HbO2, 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.

**RESULTS**

Experimental variables: 15% and 30%Hct, 0.075ml/min at 10% split ratio (Qwaste/QmetRBC). Each experiment included a control device (no magnet) and magnet device simultaneously.

The clearance efficiency of metRBCs (CmetRBC) 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 %.

\[
C_x = \left[ \frac{\text{Hct}_{\text{in}} \times \%_{\text{x,out}} - \text{Hct}_{\text{in}} \times \%_{\text{x,in}}}{\text{Hct}_{\text{in}} \times \%_{\text{x,in}}} \right] \times 100%
\]

metRBC preparation results (n=18)

- Hemoximeter analysis: 34.9±0.6%Hct, 0.2±0.0% HbO2, 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

**DISCUSSION**

GOALS

- No separation in control (no magnet)
- 0% < CmetRBC < 5% (no healthy in waste)
- SR > CmetRBC < 100%, ideal = 100% (no metRBC in outlet)

Future plans: obtain manuf. of 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

**ACKNOWLEDGEMENTS**

NIH 15R43HL110508-01A1, 3R01HL89456 and R01 HL089456-A1

**REFERENCES**