Portable optical diagnostics for early malaria detection

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ABSTRACT

Malaria remains a global health challenge, especially in the developing world. Early diagnosis of malaria infected population in low-resource areas is of great interest and need. However, the cost and the reliability of the current methods is still a fundamental concern. Here, we demonstrate a portable optical diagnostic system (PODS) for malaria screening by detecting the malaria pigment, hemozoin. In our experiments, β-hematin (a mimic for hemozoin) is used to allow for the verification of our device without the need to handle malaria-infected samples. The system is optimized and tested with spherical iron oxide magnetic nanoparticles and β-hematin in different concentrations of PEG solutions. Finally, β-hematin in whole rabbit blood is detected with this system. Detection limits of <8.1 ng/mL (corresponding to <26 parasites/µL) in 500µL of blood are demonstrated. The threshold for early stage malaria infection is 100 parasites/µL. Therefore, the present system is easily able to detect within a clinically relevant range.

Keywords: malaria, portable diagnostics

1. INTRODUCTION

Malaria remains a significant global health problem with nearly half of the world’s population living in malaria-endemic regions and more than 500,000 deaths from malaria and its complications each year. Although significant success has been achieved in malaria therapeutic development, accurate early-stage diagnosis of the disease remains a barrier to eradication, especially in low-resource areas, as treatment is nearly 100% effective when quickly and properly prescribed.[1, 2]

Despite significant investment in technology, novel, accurate, and affordable technologies for point-of-care malaria diagnosis are lacking. Light microscopy of blood smears has been the gold standard for malaria diagnosis for over a century,[1-4] and among methods currently used, it is the most reliable and sensitive. However, it is low-throughput, requires significant expertise and training, and is both labor-intensive and expensive as it requires the use of high-powered microscopes.[5-7] In recent years, antigen-based rapid diagnostic tests (RDTs) have been developed for malaria diagnosis. When compared to light microscopy, these tests are fast, easy to perform, less expensive, and do not require electricity or specific equipment or user training.[8, 9] However, they are limited in sensitivity, have a higher rate of delivering a false positive result,[10] and, recently, concerns have arisen about their stability.[11]

In this work, we designed, built, and validated a portable optical diagnostic system for malaria detection. The system is based on the detection of Hemozoin, which is a magnetic nanoparticle byproduct of the parasite. Therefore, the presence of Hemozoin is indicative of malarial infection. Unlike all other naturally occurring materials in the blood, hemozoin is paramagnetic. This property is the foundation of our magneto-optic detection system.
2.  ENGINEERING DESIGN

2.1 Hemozoin

The optical sensor system is designed to detect Hemozoin, which is a direct indicator of malaria. Hemozoin is a byproduct of the parasite formed during the intraerythrocytic growth cycle. Therefore, if found in a patient, its presence is indicative of malarial infection. Malaria parasites use hemoglobin as their primary nutrient source, leading to parasite growth and asexual replication while also generating monomeric heme, which is highly toxic to the parasite. As the parasite is unable to excrete the free heme and does not possess a heme oxygenase to recover the iron and detoxify the heme, heme is converted by the parasite in a crystallization process to form insoluble hemozoin.

Hemozoin is an ideal biomarker. It has several properties that make it unique from other, naturally occurring substances in the blood. The morphology of hemozoin varies depending on the parasite species, though the crystals typically have an elongated rod-like shape with a length ranging from 300 nm to 1 um. Additionally, it is optically opaque across the visible spectrum, it is birefringent, and it is paramagnetic.

Previous efforts have successfully used Hemozoin as a basis for a malaria diagnostic system based on a wide range of techniques including laser desorption mass spectrometry, Raman spectroscopy, cytometry, and polarization microscopy. Given the high specificity of Hemozoin as a clinical marker for malaria, all methods have demonstrated the ability to detect malaria parasitic infections at clinically relevant levels. However, similar to RDTs and blood smear imaging, these methods also rely on very complex instrumentation and highly trained personnel. Additionally, the time-to-diagnosis and the amount of sample required can be significant, further increasing the difficulty of implementing in a low-resource environment.

2.2 Beta-Hematin synthesis

To facilitate the development of Hemozoin-based detection systems, a Hemozoin-mimic called β-hematin was developed. This nanoparticle allowed researchers to develop Hemozoin diagnostics without handling malaria-infected blood samples. Notably, β-hematin has the same physical properties as hemozoin generated by P. falciparum, including the same unit crystal structure, magnetic behavior, and optical properties. The synthesis of β-hematin is well-established.

Specifically, the β-hematin synthesis used in this work relies on an acetate-mediated production route. Hemin (90 mg, Fluka) was dissolved in 10 mL of NaOH (0.1 M) and neutralized with 1 mL of HCl (1 M). To this solution, 9.25 mL of acetate buffer (9.7 M, pH 4.8) was added, and the mixture was incubated for 1 hour at 60°C. After incubation, the reaction was quenched with water, and the mixture was cooled over ice. The resulting precipitate was collected via filtration and extensively washed with water until a neutral pH is achieved. To remove any unreacted hemin, the air-dried precipitate was placed in a 15 mL Falcon tube with 1 mL of an aqueous pyridine solution consisting of 5% (v/v) pyridine, 40% (v/v) acetone, and 0.02 M HEPES (pH 7.4). This well-shaken mixture was diluted to 10 mL with water, centrifuged for 10 minutes, and the supernatant discarded. The resulting precipitate was washed with water until the supernatant was clear. Finally, the precipitate was collected via filtration and left to dry over P2O5.

Using these particles, along with control spherical iron oxide nanoparticles, solutions covering a range of concentrations are made. The solutions were in 0% and 10% PEG to evaluate the role of viscosity on the signal generation.

2.3 Device design

To develop a diagnostic system for malaria, we focused on designing a system that met relevant size, weight and power (SWaP) requirements as well as required low amounts of sample and no sample preparation or reagents. We also considered system lifetime and the system maintenance, considering the complexity of replacing broken components. This design strategy resulted in the diagnostic system shown in Figure 1. The detection mechanism is based on differential optical spectroscopy where two measurements are taken and compared: one without a magnet and one with a magnet. If there is a signal difference, then the Hemozoin is present.
Key features of the diagnostic system design include:

- By using differential optical spectroscopy, patient-to-patient variation is eliminated as a variable.
- No reagents are required.
- The only moving part in the system is the magnet on a flip mount, which can be purchased on Amazon or could be replaced by a hinge.
- The laser is a ~mW laser, similar to a red laser pointer.
- The entire system is run on a laptop (including the laser) and weigh 10lbs.

To validate the system performance, several different measurements were performed using the magnetic nanoparticle solutions and the results were compared to a predictive theory based on a combination of optomagnetic theory and mass transport.

3. RESULTS AND DISCUSSION

The results from three different experimental measurements are shown in Figure 2. Figure 2(a) and Figure 2(b) are iron oxide nanoparticles in water without and with 10% PEG. Figure 2(c) is β-hematin with 10% PEG. Several key observations can be made from these results.

First, when comparing the two data sets for the iron oxide nanoparticles (Fig 2a, b), the increase in viscosity due to the PEG significantly changed the signal generation. At the highest particle concentration, the signal change rate occurs much faster in the water than in the PEG. Additionally, background noise is much more prevalent with the PEG present. However, low concentrations (under 1ug/mL) are able to be easily detected.

In the β-hematin solutions, easily detectable signals are observed at concentrations that are comparable to clinically relevant concentrations of hemozoin in blood (Figure 2c). Notably, all measurements required only 500uL of sample and are performed with the self-contained system shown in Figure 1.[28] For both types of nanoparticles, the experimental values can be well-fit by the predictive theory.
Figure 2. Results for tests with (a) spherical Fe$_3$O$_4$ nanoparticles in water, (b) spherical Fe$_3$O$_4$ nanoparticles in 10% PEG, and (c) β-hematin in 10% PEG. Experimental results are solid lines; mathematical results are dashed lines.

4. CONCLUSIONS

In conclusion, we have developed and demonstrated a portable and self-contained malaria diagnostic based on the magneto-optic detection of hemozoin. Able to detect clinically relevant concentrations of hemozoin in whole blood samples without additional reagents, this system will enable correct diagnosis of malaria in low resource environments, potentially allowing early-stage therapeutic intervention to occur.

REFERENCES
