Development of a Cell-Free Culturing System for Liver-Stage Malaria Parasites

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BACKGROUND

Malaria is a vector-borne disease transmitted by *Anopheles* mosquitoes. Transmission begins when the mosquito takes a blood meal from an infected host, allowing the malaria parasites to replicate through a process called sporogony (Figure 1) (WHO, 2023). This process leads to the production of infectious sporozoites that collect within the mosquito's salivary glands where they can be transmitted to new hosts when the mosquito takes another blood meal (Figure 1).

The liver-stage plays a crucial role in the malaria life cycle (Figure 1) as it is an important bottleneck that leads to the asymptomatic amplification of parasite number in the infected host. However, the field is limited as there is no *in vitro* system to study the early-to-late liver-stage development of these parasites using *P. yoelii*, a mouse malaria strain that serves as a valuable model organism for studying human malaria (*P. falciparum*) (Foquet et al. 2018). The challenge lies in the inability to complete *P. yoelii* liver-stage development *in vitro*. PFO, also known as perfringolysin O, is a cholesterol-dependent protein that has the potential to form pores and selectively permeabilize the plasma membrane (Johnson et al. 2014 and Glomski et al. 2002) to aid in extracting the parasitophorous vacuole from infected cells (Figure 2) (Kleba et al. 2008). We are currently developing a protocol for deriving the parasitophorous vacuole from an infected cell using *in vitro* techniques, enabling the study of early-to-late liver-stages parasite development (Figure 3) (Kleba et al. 2008).

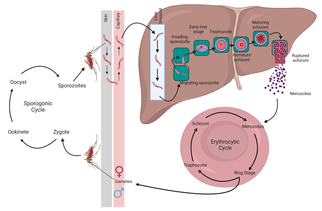


Figure 1. Life cycle of malaria parasites within infected *Anopheles* mosquitoes vector and human host. Created on BioRender (Sabrina Bacher 2022).

GOAL

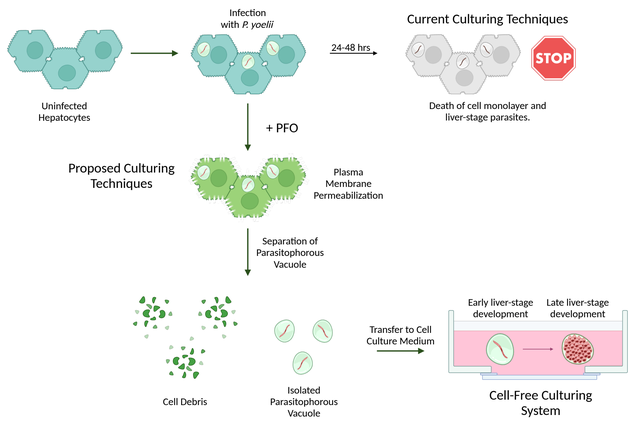
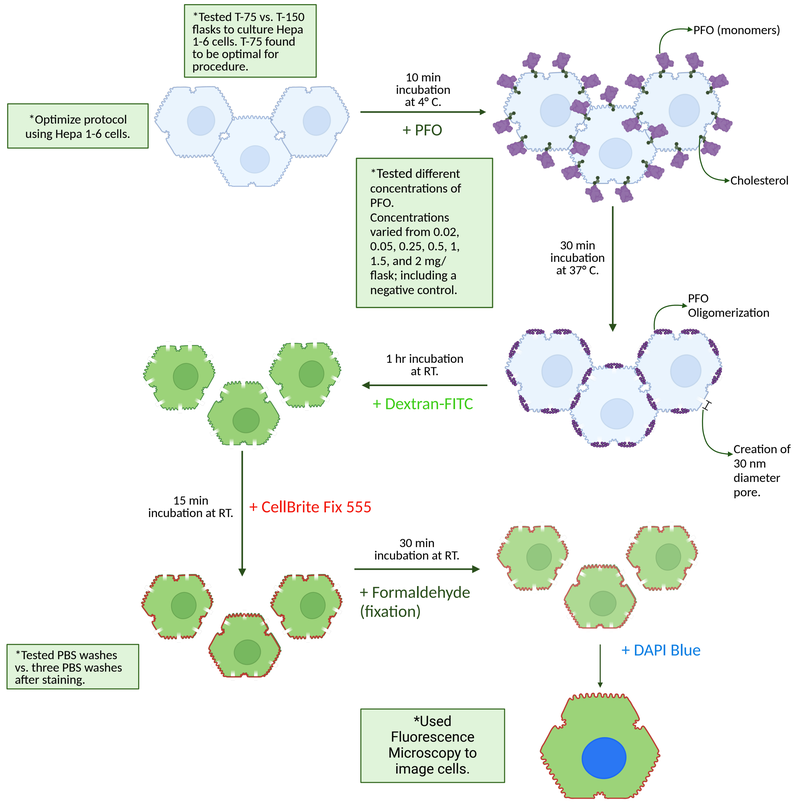


Figure 2. Project goal is to selectively permeabilize the cell membrane of *P. yoelii*-infected Hepa 1-6 cells using PFO. Isolating the parasitophorous vacuole would facilitate culturing *P. yoelii* parasites using a cell-free *in vitro* system. Created on BioRender (Sabrina Bacher 2022).

METHODS + RESULTS



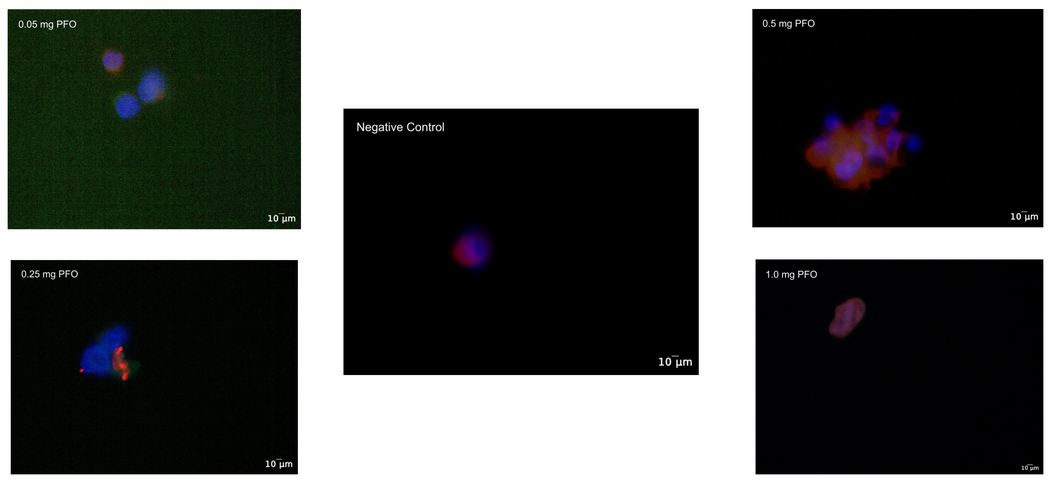


Figure 3. Fluorescent images of uninfected Hepa 1-6 cells permeabilized using PFO (1000x total magnification). Different concentrations of PFO were tested to determine the ideal range for permeabilization without inducing cell lysis (negative control, 0.05 mg, 0.25 mg, 0.5 mg, and 1.0 mg per flask are shown here). A non-specific cell membrane dye was used to fluorescently label the plasma membrane (CellBrite Fix 555, red) , dsDNA (nuclei) was fluorescently labeled using DAPI (blue) while Dextran-FITC (green) was used as an indicator of PFO-mediated permeabilization of the plasma membrane.

FUTURE DIRECTIONS

Moving forward with this procedure, we plan to alter the PFO dose dependency assay. Images (Figure 3) did not depict a clear progression of increased intracellular FITC signal when treated with increasing concentrations of PFO. Overall, our findings suggest that using less PFO results in less selective permeabilization (Figure 3). Lower concentrations of PFO generally result in lower intracellular accumulation of Dextran-FITC (green), indicating little to no permeabilization. After calibrating the PFO dosage, we plan to attempt the assay using *P. yoelii* infected cells. We will continue to optimize the use of higher concentrations of PFO to selectively permeabilize infected cells and isolate the parasitophorous vacuole to study early-to-late liver-stage development of malaria parasites *in vitro*.

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