Development of a novel real-time method to study the erythrocytic life cycle of *Plasmodium falciparum* using Quartz Crystal Microbalances

Entwicklung einer neuen Echtzeit-Methode zur Untersuchung des erytrozytärer Lebenszyklus von *Plasmodium falciparum* mit Schwingquarzbiosensoren

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This doctoral thesis was written during my work on 1.08.2006-15.02.2010 within the Biosensors Group (AG Gehring) of the Institute of Experimental and Clinical Transfusion Medicine under the guidance of Dr. rer. nat. Frank K. Gehring in cooperation with Dr. Mordmüller (Institut of Tropical Medicine, University of Tübingen). The evaluation was organized by Prof. Dr. Northoff, Institute of Clinical and Experimental Transfusion Medicine University of Tübingen, and Prof. Dr. Laufer of Institut of Pharmacy, University of Tübingen.

ABSTRACT

Development of a novel real-time method to study the erythrocytic life cycle of Plasmodium falciparum using Quartz Crystal Microbalances (QCM)

Previous studies carried out by the Biosensor Research Group of the Institute of Clinical and Experimental Transfusion Medicine at the Tübingen University had successfully demonstrated determination of various blood types through the interaction of erythrocytes with immobilised antibodies on a QCM. These studies have opened the possibility to test new applications of this technique for the study of infectious diseases. In our case, studies of the erythrocyte life cycle of *P. falciparum*, particularly during the last six hours preceding the merozoite release and studies related to reinfection of by merozoites were the focus of our investigation.

Release of malaria parasites of *P. falciparum* from infected erythrocyte at the end of their asexual erythrocyte cycle occurs approximately every 48 hours, asynchronously in parasite culture and synchronously *in vivo*. This process is poorly studied due to: (I) merozoite release is a very short event (<60 S), (II) schizont stages have high sensitivity for culture conditions like pH, medium, osmotic pressure, gas atmosphere and temperature. (III) Schizont conditions are not easy to maintain under the miscrocope, making miscroscopical real time observation of the release difficult.

The aim of the presented project was to study the release of *Plasmodium falciparum* merozoites from erythrocytes with the QCM sensor technique. In this way, the frequency shift due to the change of mass associated to the merozoite release and, on a second QCM, the reinfection of healthy erythrocytes are monitored in real time. Our QCM experiments included the following stages: (I) Adaptation and optimisation of the immobilisation of biological layers to capture the erythrocytes on the quartz. (II) Optimisation of parasite culture conditions in a QCM chamber. (III) Observation of the frequency signal both for infected and non-infected erythrocytes samples and correlation of the signal with the release of merozoites. (IV) Reinfection of healthy erythrocytes on a second quartz within one QCM system. (V) Test of inhibition of merozoite release and reinfection by antimalarial compounds.

The results showed that there was significant increase of ~1000 Hz for QCM with infected erythrocyte compared to QCM with healthy erythrocytes, where the frequency remained stable. Microscopical observation of the quartz surface at different times during the experiment (TEM and optical) demonstrated a correlation between the increase in frequency and merozoite release. At this point, approximately more than 80% of the infected erythrocytes on the quartz are involved in the release. Reinfection of new erythrocytes was observed on a second QCM. pH of the system (7.2), Temperature (37°C+/-0.1), flow of the medium (9 µL/min), sterility of the process (BactAlert), gas atmosphere (O_2 5%, residual N_2) were established to ensure parasite development and survival. External controls using flow cytometry 24 hours after the reinfection show a parasitemia percentage of >1% in the erythrocytes infected in situ.

Our results show, that the QCM technique is an appropriate and important new tool to elucidate the biology of the re-invasion process of Plasmodia.

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ABRREVIATIONS

ABRA	Acid Basic Repeat Antigen
AMA-	Apical Membrane Antigen
AO	Acridine Orange
ATCC	American Type Culture Collection
BAW	Bulk Acoustic Wave
CCD	Charge coupled device
CSA-	Chondroitin sulfate A
DARC	Duffy Antigen Receptor for Chemokines
DGs	Dense Granules
DHA	Dihydroartemisin
EBA-	Erythrocyte Binding Antigen
EPM	Erythrocyte Plasma Membrane
ER	Endoplasmic reticulum
FACS	Fluorescense Activated Cell Sorting
GA	Glutaraldehyde
GMEP	The Global Malaria Eradication Program
GPI-	Glycosylphosphatidylinositol
IC	Inhibitory concentration
ICAM-	Intracellular Adhesion Molecule
MACS	Magnetic Cell sorting
mH	Motional inductance
MR4	Malaria Research and Reference Reagent Resource Center
MSP-	Merozoite Surface Protein
MW	Molecular weight
PBS	Phosphate buffered saline
PF	Paraformaldehyde
Pf	Plasmodium falciparum
PfEMP-	P. falciparum Erythrocyte Membrane Protein
PfRBL	P. falciparum reticulocyte binding protein homolog (PfRh)
PLL	Poly-L-Lysine
PVM	Parasitophorus Vacuole Membrane
QCM	Quartz Crystal Microbalance
RAP	Rhoptry Associated Antigen
RBC	Red Blood Cells
RBCM	Red Blood Cell Membrane
RBL	Reticulocyte binding-like
RCD	Red Cell Deformability
RESA	Ring Erythrocyte Surface Antigen
Rhop	Rhoptries
RIMA	Ring Membrane Antigen
RPMI	Roswell Park Memorial Institute
RT	Room Temperature
SAMs	Self-assemble monolayers

SERA	Sera Repeat Antigen
SERCA	Sarco-endoplasmic reticulum Ca^{+}
SR	Sarcoplasmic reticulum
TBF	Thick Blood Film
TEM	Transmission Electron Microscopy
THBF	Thin Blood Film
TSMR	Thickness Shear Mode resonator
WHO	World Health Organisation

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