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Individual-based model and simulation of *Plasmodium falciparum* infected erythrocyte *in vitro* cultures

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Abstract

Malaria is still one of the most fatal diseases in the world. Development of an effective treatment or vaccine requires the cultivation of the parasite that causes it: *Plasmodium falciparum.* Several methods for *in vitro* cultivation of *P. falciparum* infected erythrocytes have been successfully developed and described in the last 30 years. Some problems arising from the current harvests are the low parasitaemia and daily human supervision requirements. The lack of a suitable model for global culture behavior makes the assay of new methodologies a costly and tenuous task. In this paper we present a model and simulation tool for these systems. We use the INDividual DIScrete SIMulation protocol (INDISIM) to qualitatively reproduce the temporal evolution of the erythrocyte and merozoite populations. Whole system dynamics are inferred by setting the rules of behavior for each individual red blood cell, such as the nutrient uptake, metabolism and infection processes, as well as the properties and rules for the culture medium: composition, diffusion and external manipulation. We set the individual description parameters according to the values in published data, and allow population heterogeneity. Cells are arranged in a three-dimensional grid and the study is focused on the geometric constraints and physical design of experimental sets. Several published experimental cultures have been reproduced with computer simulations of this model, showing that the observed experimental behavior can be explained by means of individual interactions and statistical laws.

Keywords: Individual-based model; *Plasmodium falciparum*; Simulation; Merozoite; Erythrocyte; *In vitro* culture; Malaria

1. Introduction

Malaria is still one of the most fatal diseases in the world, and it continues to be a great burden for the affected areas (WHO, 2005). Pathologic symptoms in humans are high fever and anemia, among others, and these appear due to blood infestation caused by a protozoan parasite. *Plasmodium falciparum* is one of the most deadly species. The sexual part of the parasite cycle takes place inside the human erythrocyte (RBC, red blood cell). This cycle begins with the invasion of the RBC by a single parasite form called merozoite and lasts 48 approximately hours. During the cycle, the infected RBC changes its physiology and ultrastructure, and four stages of the infection cycle are usually distinguished: ring stage, tropozoite stage, schizont stage and fragmenter stage. At the end of the parasite cycle the RBC dies and lyses, releasing from 5 to 30 merozoites that may invade other RBCs. *In vitro* cultivation of the erythrocytic stages of *P. falciparum* has been widely and usefully applied in nearly every aspect of research on malaria; it is essential for the development of an effective malaria treatment or vaccine. The current methods in use for *in vitro* propagation of the parasites are based on the *candle-jar method,* which was first developed by Trager and Jensen (1976). This method keeps RBCs incubated at 38 °C in an artificial culture medium mixed with human serum in a low oxygen atmosphere. It demands daily human supervision, as the medium must be replaced, and subcultures are required twice a week to limit culture

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infection rates. There is a demand for improving the current culturing techniques, and modeling the whole culture system behavior is taking the first steps in this direction (Jamshidi and Palsson, 2006).

Large-scale dynamic modeling of cell populations and subsequent computer simulations may provide clues for a correct description of our culture system (Henson, 2003), as it has thrown light over the global behavior of many microbial communities, such as bacteria and yeasts (Ginovart et al., 2002b, c, 2006; Prats et al., 2006). Many mathematical models for describing the dynamics of *in vivo P. falciparum* infected human RBC populations have been carried out, regarding the human immune response (Hoschen et al., 2000; McKenzie and Bossert, 1997) and the differential behavior among variants through a single infection. They have also proved to be useful for describing specific features of both *in vivo* and *in vitro* systems, such as the effect of temperature variation on the infected cells population (Kwiatkowski and Nowak, 1991) and the assessment of cross-resistance to antimalarial treatments (Noedl et al., 2001). However, models based on a mathematical description of the population dynamics of infected RBC *in vitro* cultures, comprised a set of differential equations to be applied to the whole system, have not been found.

Individual-based Models (IbM) are bottom-up approaches to the culture systems: they start from description at an individual level to infer global behavior. By using IbM, we can study different aspects of the individual model separately, as well as their cross-effects on population evolution. IbM simulations have been shown to be useful for reproducing and describing the behavior of complex systems such as microbial communities in batch cultures (Kreft et al., 1998), trophic relations in an ecosystem (Grimm, 1999), and the spreading of malarial epidemics (Gu et al., 2003), among others. INDividual DIScrete SIMulation (INDISIM) is the IbM methodology used to perform the simulation experiments of the present model (Ginovart et al., 2002a).

Our work is part of a project designed to make a description of the population dynamics of the asexual cycle of the parasite in the human blood in the mesoscopic scale. The ultimate goal of the project is to improve current experimental culture protocols.

In this paper we present a model that includes both RBC and merozoite populations in a three-dimensional space grid. Its suitability for simulating *in vitro* cultures has been checked by reproducing the experimental results obtained in several classic published experiments. The preservation of *P. falciparum* infected RBCs for 64 h performed by Pavanand et al. (1974) was used to calibrate our model, and the continuous culture of infected RBCs for 48 and 54 days performed by Jensen and Trager (1976) was used to validate it. We addressed our study to the spatial characteristics of the cultures and the evolution of the parasitaemia (the relative population of infected RBC to total RBC population). Specifically we have dealt with a collection of RBCs settled on the bottom of *in vitro* static cultures, composing the sediment called the hematocrit layer. This model is being used as an aid for the experimental task performed by the Drug Discovery Biology Group (DDBG), Diseases of the Developing World Center, from GlaxoSimthKline, R&D, Tres Cantos, Madrid.

An outlined description of the IbM is presented below, in line with the "Overview–Design concepts–Details" (ODD) standard protocol (Grimm et al., 2006). The Overview of the model consists of three elements: Purpose (Section 2.1), State variables and scales (Section 2.2) and Process overview and scheduling (Section 2.3). Design Concepts are described in Section 2.4. This section presents some general concepts underlying the design of the model and the strategic considerations taken into account to deal with the complexity of the system. Next, some details that were omitted in the overview are specified. Initial values for the characteristic variables and parameters of the model are presented in Section 2.5, and the external constraints introduced in our model are depicted in Section 2.6. Section 2.7 describes in more detail the submodels that represent the processes listed in Section 2.3. Section 3 is addressed to the particular results obtained by the simulation experiments. Section 3.1 presents the experimental data and sets in real systems, while the input data to carry out the corresponding virtual experiments are presented in Section 3.2. Section 3.3 presents the simulation results. Section 4 expounds an analysis of the results and the model, and Section 5 concludes assessing the outcomes of this work.

2. Description of the model

2.1. Purpose

The first aim of the current work is to uncover the physical processes that determine the evolution of healthy and infected RBC populations within an *in vitro* culture of *P. falciparum* infected cells. In particular, it tackles the effect of the limitations on the diffusion of important solutes for the RBC metabolism, and the role of the process of extracellular parasite spreading through the hematocrit layer. We also focus on the macroscopic physical and geometric constraints to which experimental designs are subjected, and on the effect of external manipulation protocols on the population dynamics. The ultimate goal of our work is to improve current experimental protocols.

2.2. State variables and scales

The model proposes two entities: individual RBCs and the culture environment. The description of the culture system is discrete both in time and space: all actions occur at constant intervals called time steps, and all entities are characterized by a set of integer numbers. Individuals are spread over a whole number of spatial cells that form