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Individual Based Model and simulation of *Plasmodium falciparum* Infected Erythrocytes *in vitro* cultures

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Abstract

Several methods for in vitro cultivation of *Plasmodium falciparum* infected erythrocytes have been successfully developed and described in the last thirty years. Some problems arising from the current harvests are the low parasitaemia per medium expenses and frequent human supervision requirements. The lack of a suitable model for the culture behavior makes the essay of new culture methodologies a costly and tenuous task. New culture protocols can make easier the research towards new antimalarial drugs. In this paper we model and simulate the culturing of parasitized red blood cells according to the current "Malaria Research and Reference Reagent Resource Center (MR4)" protocols in use. We use the Individual Based Model methodology to reproduce the temporal evolution of the erythrocyte and merozoite populations. We set the rules of behavior for each individual cell (healthy "RBC" and infected "IRBC" erythrocytes): uptake, metabolism, infection process and intraerythrocytic parasite evolution, as well as the rules for the culture system: geometric characteristics (flask dimensions, medium volume, local cell concentration), medium composition and substance diffusion, and external manipulation (dilution, subculturing, discrete and continuous agitation). Parameters used for the simulations are based on experimental data in the literature, or taken from published models. We compare the behavior of the simulated model harvest with that of the real cultures carried out in the DDW Research Center GlaxoSmithKline laboratories. We have found the model suitable for simulating the collective evolution of these cultures, as well as a good tool for describing the effect of the experimental protocols on the infected erythrocyte population. We show that many observed phenomena such as the different growth rates in static or agitated cultures or the need of medium renewal can be explained by means of the local interactions between individuals and medium. We propose experimental protocols to improve malarial in vitro harvests

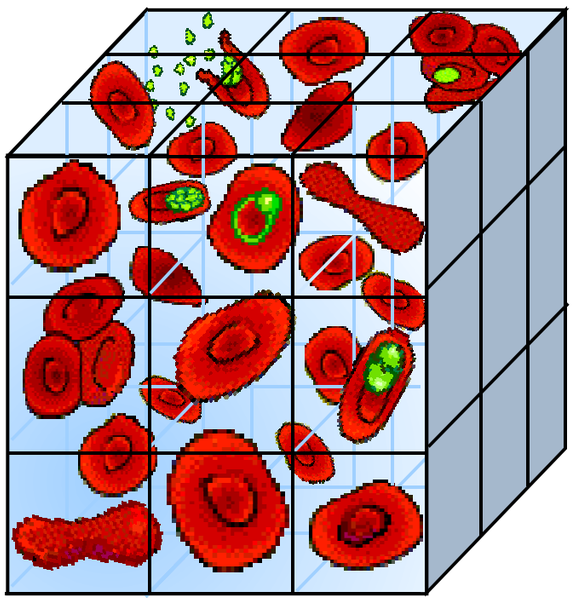
Model

INDISIM

→Individual based Model
→Discrete in space and time (Time step=6 minutes)
→Controls individual behavior, medium conditions and protocols.
→Allows variability among the cellular population.
→ Reproduces stochastic processes
→Useful for isolating the different importance of biologycal mechanisms
→Proven to be good for describing bacterial communities (1,2)

SIMULATON SPACE

→Represents a small fraction of the culture system of $5 \cdot 10^{-6}$ml volume; contains 40000 RBCs
→Simulates the hematocrit layer. Boundary conditions are periodic in the horizontal directions, open to substance diffusion through the upper surface and closed through the basis.
→Is divided into 64000 cubic spatial cells, each one containing up to two RBCs. Individual actions are local: they affect the occuped cell and first neighboring spatial cells at most.
→ Density varies for suspended cultures.



INDIVIDUALS

RBCs
→ Act at each time step
→ Need to uptake glucose, produce and dump lactate into the medium.
→ Age- and time in culture-decreasing invasion susceptibility.
→ Death comes after scarce of nutient, excess of age or time in culture, or due to fragility.

IRBCs
→ Four infection stages: ring, tropozoite, schizont and fragmenter.
→ Stage-depending fragility and metabolic needs.
→ Lyses at the end of infection cycle, releases merozoites.

CULTURE MEDIUM

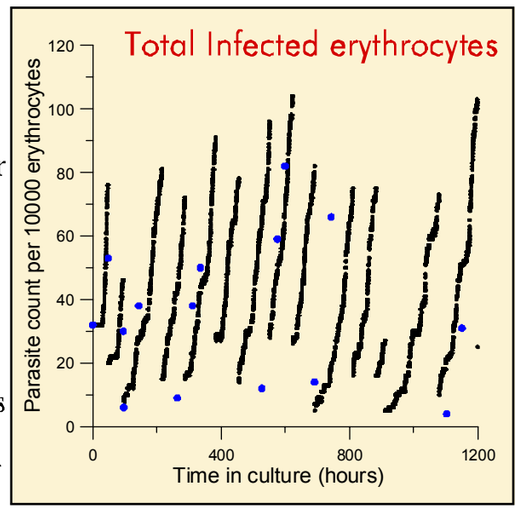
MEROZOITES
→ Scattered egress from IRBCs at the same spatiall cell where lysis occurs and at nearest neighboring cells.
→ Remain during four time steps in the culture medium.
→ No active, nor diffusive motion, merozoites fall due to gravity.
→Invade RBCs at own spatial cell.

SOLUTES
→ Local concentration varies due to red blood cells' metabolic action.
→ Diffusion smooths out concentration gradients
→Frequent medium renewal.

Results

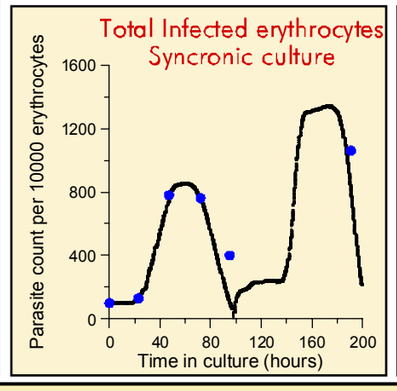
CALIBRATION

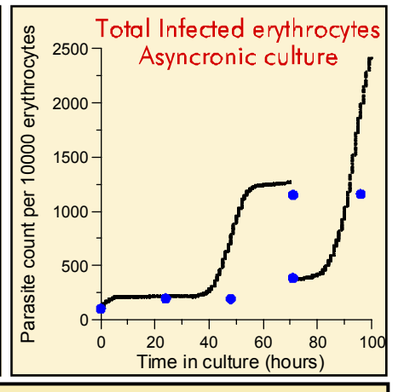
→Program ruled by 51 parameters.
→Values for the parameters taken from theoretical published models or from direct experimental measures (a detailed description of the model is in process).
→Some values not found, then adjusted by fitting simulation results to experimental measures.
→ We use data from the experiments performed by K.Pavanand (3), and Jensen & Trager (4). We fit data for each stage and for the total infected population evolution.



REPRODUCTION OF EXPERIMENTAL RESULTS

DDW Research Center GlaxoSmithKline Experiments follow the MR4/ATCC protocols (5) , use same parasite strain and different inoculums and serum. Simulations adjust inoculum syncronism and protocol.

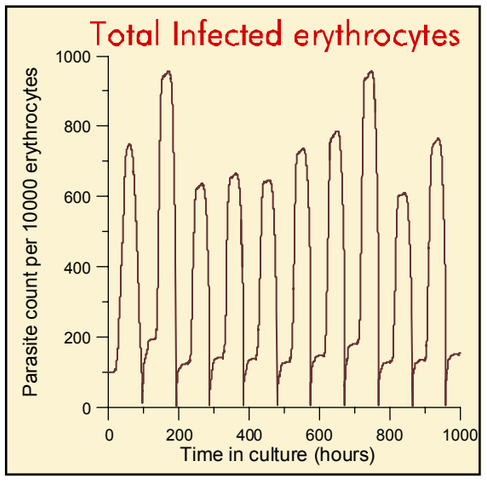




Experimental parasitaemia count Simulation parasitaemia count

EXAMPLE OF A PROPOSED PROTOCOL

Figure shows the simulation of an automatic culture system, subculturing is automatic and requires no human supervision. First two dilutions at 1/4, afterwards at 0.97



Conclusions

1.- With the current methodology we can model and simulate *in vitro* cultures. 2.- 3D simulations show us the main relevance of :ge ometric factors concerning parazite propagation, and the importance of the velocity of degradation of *in vitro* erythrocytes. 3.- They also show us that there is no local medium degradation (scarce of glucose) due to diffuson limitations, in most of the cultures. 4.- We are able to correctly reproduce current experimental cultures and propose new protocols

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(3) **K.Pavanand *et al.***, J. Parasitol. **60** (3) 537-539 (1974)
(4) **W. Trager, J.B. Jensen.**, Science **193** 673-675 (1976).

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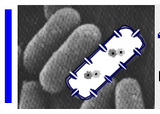

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