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ORIGINAL PAPER

Analytical modeling of the mechanics of early invasion of a merozoite into a human erythrocyte

Tamer Abdalrahman[1] · Thomas Franz[1,2]

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Abstract In this study, we used a continuum model based on contact mechanics to understand the mechanics of merozoite invasion into human erythrocytes. This model allows us to evaluate the indentation force and work as well as the contact pressure between the merozoite and erythrocyte for an early stage of invasion ($\gamma = 10\%$). The model predicted an indentation force of $1.3e^{-11}$N and an indentation work of $1e^{-18}$J. The present analytical model can be considered as a useful tool not only for investigations in mechanobiology and biomechanics but also to explore novel therapeutic targets for malaria and other parasite infections.

Keywords Cell mechanics · Malaria · Merozoite · Erythrocyte · Contact mechanics · Parasite invasion

1 Introduction

Malaria is one of the most common infectious diseases and a great public health problem worldwide, particularly in Africa. It is transmitted by an infected female *Anopheles* mosquito. About half of the world's population lives in areas where people are at risk of getting malaria through the bites of infected mosquitoes.

Extensive effort has concentrated on understanding the procedure of invasion for every phase of the disease with a view to developing novel therapeutics or antibodies to forestall

✉ Tamer Abdalrahman
tamer.abdalrahman@uct.ac.za

Thomas Franz
thomas.franz@uct.ac.za

[1] Department of Human Biology, Division of Biomedical Engineering, Faculty of Health Sciences, University of Cape Town, Observatory, Cape Town, South Africa

[2] Bioengineering Science Research Group, Engineering Sciences, Faculty of Engineering and the Environment, University of Southampton, Southampton, UK

or treat the disease [1]. The process by which the parasite, called merozoite, invades an erythrocyte involves mechanical forces, as the parasite needs to penetrate the plasma membrane of the host cell. A detailed description of invasion of merozoite based on the internal molecular motor beyond the scope of this article is available elsewhere [2]. In spite of considerable advances, a systematic and mechanistic understanding of the parasite entry into host cells through invagination is still elusive. Invagination is a complex process, which involves ligands and likely hundreds of cellular proteins, such as parasite receptors, multifactorial machineries, and signaling pathways associated with clathrin, actin, dynamin, caveolin, and microtubule networks to name a few [3].

The first research on the biomechanics of malaria was performed in 1971 by Miller et al. [4, 5]. They examined suspensions of monkey erythrocytes (red blood cells, RBC) infected with two different strains of the malarial parasites, using a viscometer and cell-filtration technique. Their outcomes were the first to indicate that infection by the parasites could weaken the deformability of erythrocytes and, consequently, the biorheological properties of blood. Cranston et al. [6], in 1984, utilized a rheoscope to examine the deformability of individual malaria-infected erythrocytes at various phases of infection. Similarly, Suwanarusk et al. [7] utilized a laminar–shear flow system to assess the deformability of erythrocytes at different stages of infection. In recent years, research has focused on the mechanistic understanding of the process of erythrocyte invasion by the blood stage merozoites through investigating the force that is required for the entry of the parasite into the host cell.

The binding energy between the merozoite and the erythrocyte is the key for successful parasite entry. While the initial contact between the merozoite and the erythrocyte is arbitrary, for successful invasion the merozoite effectively re-orients itself using actin-myosin motors, to get its apical end in contact with the cell membrane [8]. Ligands attach to the receptors on the erythrocyte membrane to form an electron-dense thickening of the erythrocyte membrane at the nexus of erythrocyte-merozoite contact. The energy released due to the contact of merozoite surface and the enveloping erythrocyte membrane must be sufficiently large that the completely wrapped state of the parasite corresponds to the lowest energy state. The early stage of invasion of an erythrocyte by a merozoite is therefore based on the transformation of chemical energy into mechanical work. The chemical energy is released by the binding of ligand molecules, distributed over the parasite surface, to receptor molecules expressed on the cell membrane. This chemical energy is converted to work of elastic deformation of the plasma membrane and the entire body of the host cell. Based on energy transformation at the early stage of the invasion process, the densities of ligands on the parasite and receptors on the host cell are the governing parameters of the contact force and the pressure distribution between parasite and host cell. Other factors affecting the mechanical interaction between the merozoite and the erythrocytes are the elastic modulus of cytosol and cytoskeleton and the modulus of rigidity of the plasma membrane of the erythrocyte. These mechanical processes of the invasion are, however, poorly understood. The invasion process of merozoite is complex and rapid. The mechanics of the invasion has been studied recently by [2, 9]. Because erythrocyte invasion is an obligate part of the parasite's life cycle, blocking the invasion prevents parasite growth, making invasion an attractive anti-malaria target. The design of anti-malarial targets can be regulated as anti-adhesion intervention. This requires a detailed understanding of the adhesion energy and how this energy is utilized during the invasion process.

The present work, hence, aimed at developing an analytical approach based on contact mechanics to characterize the effect of cell stiffness on forces and pressure at the early stage of invasion, hence, calculations were limited to $\gamma = 0.1$.

2 Method

The dimension of a human erythrocyte is roughly 7.5 to 8.7 μm in diameter and 1.7 to 2.2 μm in thickness [10]. The physical parameters of a merozoite were: width W = 1.40 ± 0.06 μm, length L = 1.98 ± 0.08 μm, volume V = 1.71 ± 0.15 μm^3, and surface area A = 8.06 ± 0.72 μm^2 [9]. Due to the egg-shape profile of the parasite, the profile of the apical side can be represented by a power law function as

$$y(r) = cr^n \tag{1}$$

where r, n, and c are the radial distance, the shape index, and the shape coefficient, respectively. Based on the length and width of the parasite, the profile is approximated to a parabolic shape with $n = 2$ and $c = 3$.

Here, the parasite and the host cell are modeled as a rigid indenter with an arbitrary profile in contact with half-space as illustrated in Fig. 1. The indentation problem of a rigid axisymmetric indenter of arbitrary shape and a linear elastic half space has been solved by Sneddon [11]. The contact theory of Sneddon [11] is applied here to describe the mechanics during the early stage of invasion of a merozoite into a human erythrocyte. The idealized merozoite is simulated as an asymmetrical egg-shaped rigid particle [9].

The combined elastic modulus of the merozoite-erythrocyte contact pair is defined as;

$$E^* = \frac{E_c}{1 - \nu_c^2} \tag{2}$$

where E_c and ν_c are the elastic modulus and Poisson's ratio of the cell, respectively [11]. As the contact between the parasite and the cell is initiated, the contact force develops during the early stage of invasion. By applying the Sneddon model, the invasion depth is given by [11]:

$$\delta = \int_0^1 \frac{y'(x)}{\sqrt{1 - x^2}}. \tag{3}$$

From Eqs. (1) and (3), the invasion depth can be formulated as

$$\delta(a) = \frac{\sqrt{\pi}}{2} \frac{n\Gamma(n/2)}{\Gamma\left(\frac{n+1}{2}\right)} ca^n, \tag{4}$$

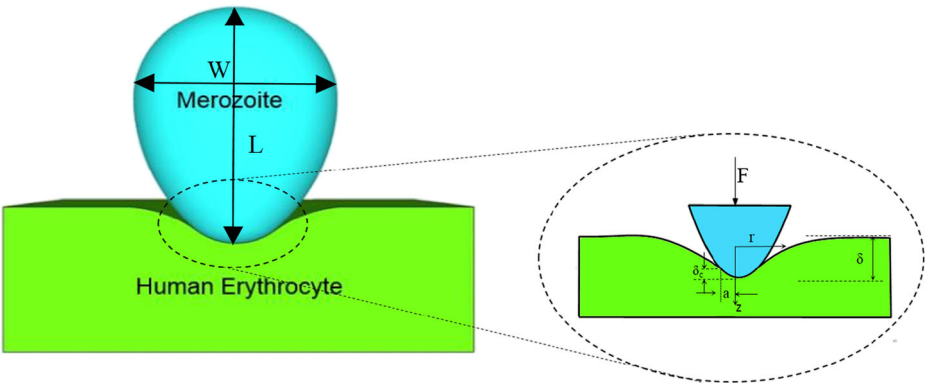

Fig. 1 The merozoite-erythrocyte contact model. The contact energy produces an equivalent parasite indentation force F, which is utilized to deform the cell where a is the contact radius and δ is invasion depth

where a is the contact radius between the parasite and the cell. The depth of parasite invasion can be expressed by the contact depth

$$\delta_c = ca^n \tag{5}$$

in the form of

$$\delta = D(n)\delta_c \quad with \quad D(n) = \frac{\sqrt{\pi}}{2}\frac{n\Gamma(n/2)}{\Gamma\left(\frac{n+1}{2}\right)}. \tag{6}$$

The invasion force can be obtained as

$$F = 2E^*\int_0^a D(n)[y(a) - y(r)]dr. \tag{7}$$

By replacing the power law function in Eq. (1), we obtain

$$\begin{aligned} F &= 2E^*\tfrac{n}{n+1}D(n)c\,a^{n+1} = E^*\sqrt{\pi}\tfrac{n\Gamma\left(\frac{n}{2}+1\right)}{\Gamma\left(\frac{n+3}{2}\right)}c\,a^{n+1} \\ &= 2E^*\tfrac{n}{n+1}\left[\frac{2}{\sqrt{\pi}}\frac{\Gamma\left(\frac{n+1}{2}\right)}{n\Gamma\left(\frac{n}{2}\right)}\frac{1}{c}\right]^{\frac{1}{n}}\delta^{\frac{n+1}{n}}. \end{aligned} \tag{8}$$

The work of the initial indentation of the parasite into the red blood cell w is

$$w = \int F\,d\delta = \int 2E^*\frac{n}{n+1}\left[\frac{2}{\sqrt{\pi}}\frac{\Gamma\left(\frac{n+1}{2}\right)}{n\Gamma\left(\frac{n}{2}\right)}\frac{1}{c}\right]^{\frac{1}{n}}\delta^{\frac{n+1}{n}}\,d\delta. \tag{9}$$

The contact pressure distribution under the parasite is given by [12]

$$P(r) = -\frac{E^*}{\pi}\int_r^a g(x)(x^2 - r^2)^{-1/2}dx, \tag{10}$$

where the auxiliary function g(r) is introduced by

$$\begin{aligned} g(r) &= \frac{-d}{dr}(r\int_0^r y'(x)(r^2 - x^2)^{-1/2}dx) \\ &= \frac{-d}{dr}(c\,n\,r\int_0^r x^{n-1}(r^2 - x^2)^{-1/2}dx). \end{aligned} \tag{11}$$

The integration part in Eq. (11) can be obtained by a Beta integral solution, where $x^2 = r^2t$ and $x = r\sqrt{t}$ will be substituted in Eq. (11):

$$\int_0^r x^{n-1}(r^2 - x^2)^{-1/2}dx = \frac{r^{n-1}}{2}\int_0^1 t^{\frac{n-2}{2}}(1-t)^{-1/2}dt \tag{12}$$

where

$$\int_0^1 t^{\frac{n-2}{2}}(1-t)^{-1/2}dt = \beta\left(\frac{n}{2}, \frac{1}{2}\right) \tag{13}$$

and

$$\beta\left(\frac{n}{2}, \frac{1}{2}\right) = \frac{\Gamma\left(\frac{n}{2}\right)\Gamma\left(\frac{1}{2}\right)}{\Gamma\left(\frac{n+1}{2}\right)}. \tag{14}$$

By substituting Eq. (14) into Eq. (11), we obtain;

$$g(r) = -n^2c\,\frac{r^{n-1}}{2}\frac{\Gamma\left(\frac{n}{2}\right)\Gamma\left(\frac{1}{2}\right)}{\Gamma\left(\frac{n+1}{2}\right)}. \tag{15}$$

From Eq. (15) and (10), the pressure between the parasite and the cell is given by

$$P(r) = \frac{n^2 c}{2} \frac{\Gamma\left(\frac{n}{2}\right)}{\Gamma\left(\frac{n+1}{2}\right)} \frac{E^*}{\sqrt{\pi}} \int_r^a x^{n-1} (x^2 - r^2)^{-1/2} dx. \tag{16}$$

Here we introduce the invasion index $\gamma = \delta/\mathrm{L}$ as invasion depth normalized to the length of the merozoite. The contact energy between merozoite and erythrocyte is proportional with their contact area A_c and can be expressed by

$$E_{adh} = \omega A_c, \tag{17}$$

where ω is the adhesion energy per unit area of contact and can be given by

$$\omega = f \rho, \tag{18}$$

where ρ is the ligand complex density in the contact zone and f is the free energy of a single receptor. The latter can be defined by

$$f = -K_B ln K \tag{19}$$

where K is the equilibrium constant of the receptor and K_B is Boltzmann's constant.

The merozoite has a gradient of the adhesion strength on its surface [9], so that ω is calculated from the value of adhesion strength at the apex side [9].

The contact area can be given as a function of contact radius (a) and invagination displacement (δ_c):

$$A_c = \frac{\pi}{6} \frac{a}{{\delta_c}^2} \left((a^2 + 4{\delta_c}^2)^{\frac{3}{2}} - a^3 \right). \tag{20}$$

3 Results

The analysis of the mechanics of merozoite invasion is important for the understanding of the factors that can contribute to the invasion process of malaria parasite into human erythrocytes. Based on contact mechanics, Eqs. (4), (8), (9) and (16) are developed which, when applied to elastic materials, can provide a description of the mechanics of early stage invasion of a merozoite in an erythrocyte.

The indentation force, the work consumed in elastic deformation, and the pressure distribution between the merozoite and erythrocyte were calculated during the early phase of invasion. In addition, the effects of a cell's elastic modulus on the indentation force were investigated.

Figure 2 provides the variation of the indentation force with varying indentation depth expressed as invasion index, for different values of the elastic modulus of the erythrocyte. Figure 2a shows a non-linear correlation between the invasion force and the invasion index which was obtained from Eq. (8). Figure 2b illustrates the parasite indentation force versus the invasion index for various values of the elastic modulus of the cell. It can be recognized that the indentation force increases linearly with the elastic modulus of the cell as stipulated from Eq. (8).

The mechanical work utilized in the elastic deformation of the cell membrane with underlying cytosol for an increasing invasion index is provided in Fig. 3a. The prediction of the work of early-stage invasion is obtained by Eq. (9), where the invasion depth (δ) is substituted with the product of original length of the parasite and the invasion index, $L\,\gamma$. As

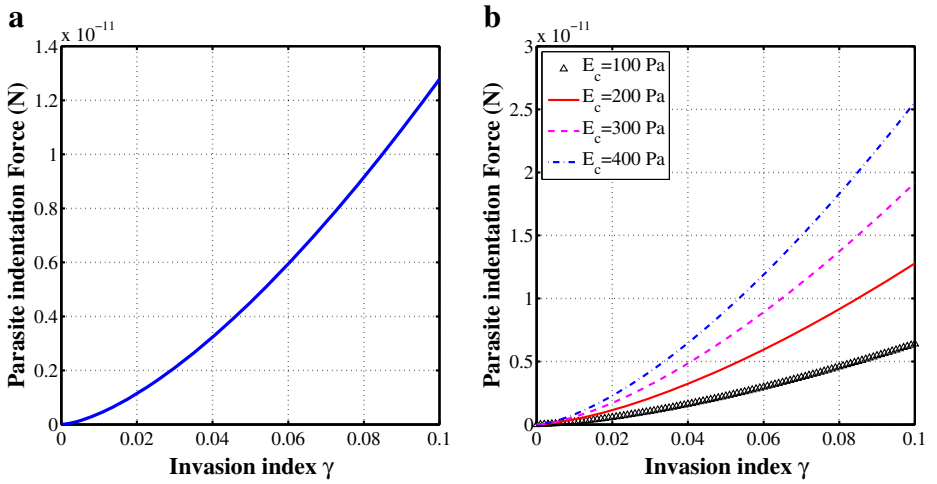

Fig. 2 Model predictions of indentation force vs. invasion index with (**a**) cell stiffness $E_c = 200$ Pa [13] (**b**) different cell stiffness

shown in Fig. 3a, the variation of the invasion work versus invasion index has a nonlinear relationship. The distribution of the contact pressure between merozoite and erythrocyte is illustrated in Fig. 3b for different values of the invasion index. The contact pressure is equal to zero at radial distance larger than the parasite–cell contact radius ($r > a$), and increases for $r = a$ to $r = 0$.

The contact energy is plotted versus the invasion index in Fig. 4, indicating a linear relationship between the two parameters. This relationship is obtained by substituting Eq. (20) into Eq. (17). The adhesion energy calculated up to $\gamma = 0.1$ is shown in Fig. 4.

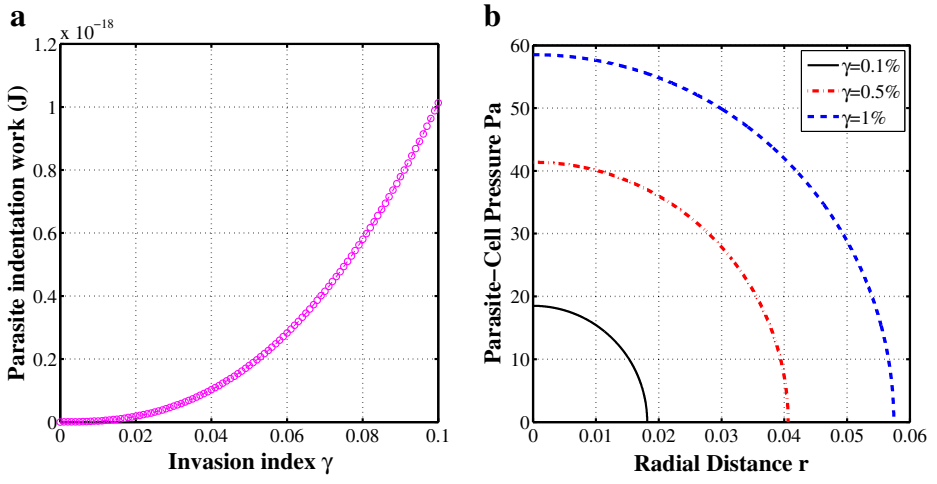

Fig. 3 Model predictions of (**a**) indentation work vs. invasion index; (**b**) contact pressure distribution vs. radial distance. The elastic modulus of host cell is assumed to be 200 Pa

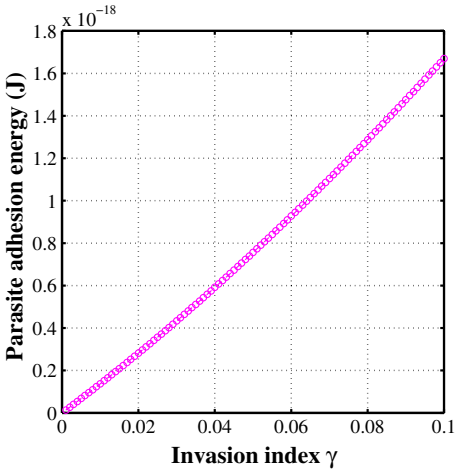

Fig. 4 Total contact energy, E_{adh}, of the parasite and host cell interaction as a function of the normalized parasite invagination depth

4 Discussion

The main objective of this study is to show how the adhesion energy contributes as a source in the invasion process of malaria parasites. The study introduced a model based on contact mechanics theory to estimate the parasite indentation force, and the pressure distribution of contact between the merozoite and the erythrocyte during the early stage of invasion. Based on the contact mechanics formulation, the model also allows investigating the effects of the mechanical properties of the cell on the merozoite invagination. In this analytical model, we utilized Sneddon's formulation for contact between an axisymmetric indenter and an elastic half space.

The model predicted that the merozoite indentation force and work during the early stage of invasion are equal to 13 pN and 1 aJ, respectively. The predicted invasion force is in reasonable agreement with the invasion force generated by myosin motors [9, 14, 15]. Based on Eq. (8), the relation between the indentation force and the elastic modulus of the host cell is linear. The effect of the elastic modulus of the erythrocyte has been shown in Fig. 2b. The work done by the indentation force at the early stage of the invagination process, found to be equal to $1e^{-18}$J at $\gamma = 0.1$, is shown in Fig. 3a, and the maximum value of contact pressure has been obtained at r = 0, i.e., the center of the contact area, as shown in Fig. 3b. The adhesion energy (Fig. 4) predicted by the model is higher than the work required for the elastic deformation of the cytoplasm during indentation (Fig. 3a) at any indentation depth. This indicates that only a part of the adhesion energy E_{adh} is utilised for cytoplasmic elastic deformation whereas the remaining part will be used for other mechanical processes, most notably the bending deformation of the cell membrane. Values reported in the literature for the free energy f and ligand complex density ρ show substantial variations [16] and are limited by high experimental uncertainties [17]. As such, the precise calculation of the adhesion energy (see Eq. (18)) is not feasible at this stage.

There are some limitations with the present model. Firstly, it does not consider individual subcellular components such as the cell membrane or cytoskeleton [18–20]. These components will be considered in future updates of this model by utilizing multi-scale modeling. Multi-scale modeling is a common method used to consider the effect of mechanical properties of subcellular components on the global constitutive behavior of cellular deformation. Secondly, the viscoelastic phenomena of cell deformation are not considered in this

model. Equations (1)–(20) are obtained based on small elastic deformations, so that the current model is valid only for the mechanics of invagination at an early stage of the invasion process, hence the calculations were limited to $\gamma \leq 0.1$. Despite the current limitations, the present analytical model can be considered as a useful tool not only for investigations in viral and cellular mechanobiology or biomechanics but also to explore new avenues for novel therapeutic targets for malaria. The mathematical model was developed to provide an accurate description of the process of early merozoite invagination. One novel treatment approach for malaria (and other diseases relying on invagination) aims to inhibit the releases of adhesion energy below the level required for the deformation of the cell during invasion. Quantitative data on invasion mechanics such as provided from the current model may greatly facilitate translation of such approaches, as well as the development of new drugs and drug delivery systems. Recent experimental work quantified the strength of interactions between merozoites and erythrocytes (utilizing laser optical tweezers) and abnormal stiffness changes in cells due to different pathological conditions, e.g., cancers and malaria (using atomic force microscopy) [21, 22]. Such mechanobiological observations should be considered in therapeutic interventions, and can be useful for drug delivery into infected cells. The presented analytical model is an example of how theoretical mechanobiology can provide a fundamental framework for studying the effects of different parameters on biological phenomena.

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Compliance with Ethical Standards

Conflict of interests The authors declare that they have no conflicts of interest.

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