Evidence of chemotaxis by quantitative measurement of the force vectors of *Trypanossoma cruzi* in the vicinity of the *Rhodnius prolixus* midgut wall cell

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ABSTRACT

In this work we used a methodology to study chemotaxis of *Trypanossoma cruzi (T. Cruzi)* in real time using an Optical Tweezers system. Trapped beads were used as a force transducer for measuring forces of the same order of magnitude as typical forces induced by flagellar motion. Optical Tweezers allowed real time measurements of the force vectors, strength and direction, of living parasites under chemical or other kinds of gradients. This seems to be the ideal tool to perform observations of taxis response of cells and microorganisms with high sensitivity to capture instantaneous responses to a given stimulus. We applied this methodology to investigate the *T. cruzi* under distinct situations: the parasite alone and in the presence of its insect-vector *Rhodnius prolixus (R. prolixus)*.

Keywords: Optical tweezers, chemotaxis, parasite-vector interaction, force

1. INTRODUCTION

One of the fundamental goals in parasitology is the fully understanding of parasite-host cell interactions. This is still more important when it comes to non-curable diseases, like Chagas disease caused by *T. cruzi*. Chaga's disease is present in 18 countries of the American continent with prevalence of 16 million cases, with 4.8–5.4 million people exhibiting clinical symptoms, an annual incidence of 700,000-800,000 new cases, and 45,000 deaths due to the cardiac form of the disease. At present, estimates indicate an infection prevalence of 13 million, with 3.0–3.3 million symptomatic cases and an annual incidence of 200,000 cases in 15 countries [1].

Interactions between *T. cruzi* and its insect-vector, *R. prolixus*, begin when the insect ingests trypomastigotes forms from blood of the infected vertebrate host. Once in the gut, the parasites multiply in epimastigotes forms and migrate to hindgut where the metacyclogenesis takes place. At this point, *T. cruzi* is capable to infect human being and other mammals. This whole cycle starts when the parasite detects the target cell and moves towards it. The process where cells and microorganisms direct their movements according to certain chemical gradients in their environment is known as chemotaxis. Chemotaxis is not the only kind of taxis of microorganisms. They also sense and respond to temperature, osmotic pressure, pressure, light and other parameter gradients involved in their survival.This is important for the microorganisms, such bacterias, to find food (for example, glucose) by swimming towards the highest concentration of food molecules, or to flee from poisons (for example, phenol) [2]. In multicellular organisms,

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chemotaxis is critical to early (e.g. movement of sperm towards the egg during fertilization) and subsequent phases of development (e.g. migration of neurons or lymphocytes) as well as in normal function. In addition, it has been recognized that mechanisms that allow chemotaxis in animals can be subverted during cancer metastasis. Cell signalization is responsible for the chemotatic activity and many chemical receptors present mostly on cell membrane are involved in this kind of taxis [3].

There are many studies in the literature on chemotaxis, and other kind of taxis, such as osmotaxis, performed by cells and microorganisms. Chemotaxis, for example, has been extensively studied from two points of view: (1) a black box point of view where the response is observed as a function of the stimulus and (2) a biochemical point of view where the biochemical reactions triggered by the receptors are observed [4; 5; 6]. The majority of the work on chemotaxis is on leukocytes, which have a kind of slow taxis based on crawling, unlike the taxis performed by bacteria and protozoa [7, 8, 9]. For parasites there are more studies on the chemotaxis of bacteria than that of protozoa [10; 11; 12]. Some methods are commonly used to study microorganism chemotaxis. The capillary assay, first created by Pfeffer [13] and later improved by Adler [14] is the most commonly used method, especially for quantitative analysis. The methodology developed by Adler was adapted by Oliveira and applied to study the chemotaxis of promastigote forms of leishmanias. This kind of methodology is quantitative, but the measurements are based only on the number of cells found near the end of the higher concentration gradient capillary. Law [4] improved the methodology, and mathematical treatment became easier to carry out than in previous works, but the parameter measured was the same as in Adler's work. Another methodology, introduced by Barros [12] to study the chemotaxis of leishmania, was based on measurement of the mean time of straight line movement. Movement in a straight line is defined here as the absence of abrupt changes of direction.

In order to clarify the whole infection process it is necessary to understand how the parasite can detect the cells. In the interaction envolving the parasite and the perimicrovillar membrane occurs attachment between molecules from both [15]. Quantitative measurements of the parasite chemotaxis in presence of *R. prolixus* perimicrovillar membrane can elucidate some points of this interaction. For a better understanding of chemotaxis it is necessary to know not only the sense and direction of the parasite's force but also its intensity. We believe that chemotaxis drives these protozoans towards the midgut cells, therefore the aim of this research is to develop a methodology to study microorganisms chemotaxis in real time using Optical Tweezers. This would be the ideal tool to perform black box observations of the taxis response of cells and microorganisms with high sensitivity to capture instantaneous responses to a given stimulus. Forces involved in the movement of unicellular parasites are very small, about the same order of magnitude as the forces generated in an optical tweezers. Optical Tweezers rely only on light radiation pressure to trap and manipulate microscopic particles. It is a fast, contactless, nondestructive and highly sensitive force measurement tool capable of measuring forces ranging from tens of femto-Newtons to hundreds of pico-Newtons.

2. EXPERIMENTAL SYSTEM

The experimental system used in this work is shown in Figure 1. We used an Olympus IX-81 inverted microscope. A Prior Scientific, model ProScan translation stage was used to move the specimens in the x-y direction, while the z-translation was performed with the IX-81 built in system. The propulsion forces of the flagellum of the protozoan were measured with an Optical Tweezers by the movement of a 9 μm polystyrene bead, connected to the parasite, as a force transducer. This size is about the same size of the parasites, and it was chosen to avoid the direct trap of the parasite, instead of the bead. In this way only the bead scatters the light and not the parasite itself.

The bead was trapped with a Nd:YAG laser (1064 nm) using an Olympus high numerical aperture objective (1.4 60x oil immersion). We assume a geometrical optics model to calibrate the force as a function of the bead displacement from the equilibrium position. The displacement of the bead due to the parasite's flagellum propulsion was then measured to determine the numerical values for the optical force and, consequently, for the force of the parasite. Previous calibration of this procedure against hydrodynamic force showed good results [16].

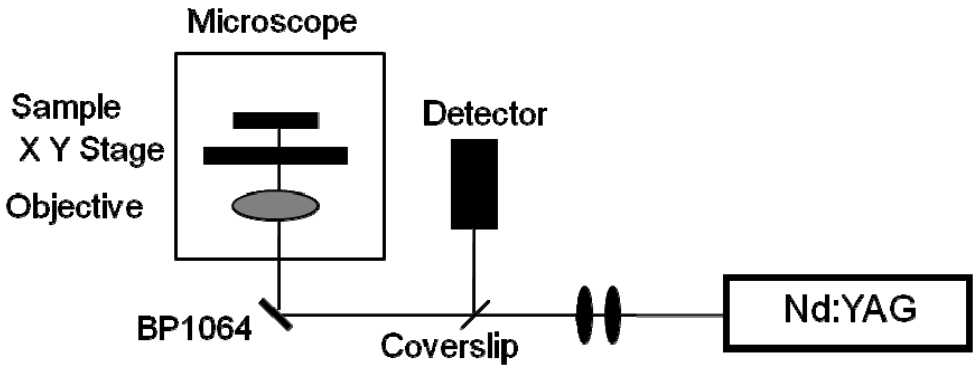

Fig1. Experimental system

The x and y displacements of the bead were obtained by measuring the scattered light of the optical tweezers laser with a quadrant detector (QP506SD2 - Pacific Sensor Incorporated) positioned in the back focal plane of the microscope. A coverslip in the light path reflected the scattered light to the detector and signal was sent to an oscilloscope (Tektronix DPO 7104). The voltage vs displacement calibration was performed by measuring the voltage as a function of the bead position controlled with a translation stage.

3. RESULTS

Figure 2 shows the calibration curves obtained. This calibration links the bead displacements with the scattered light.

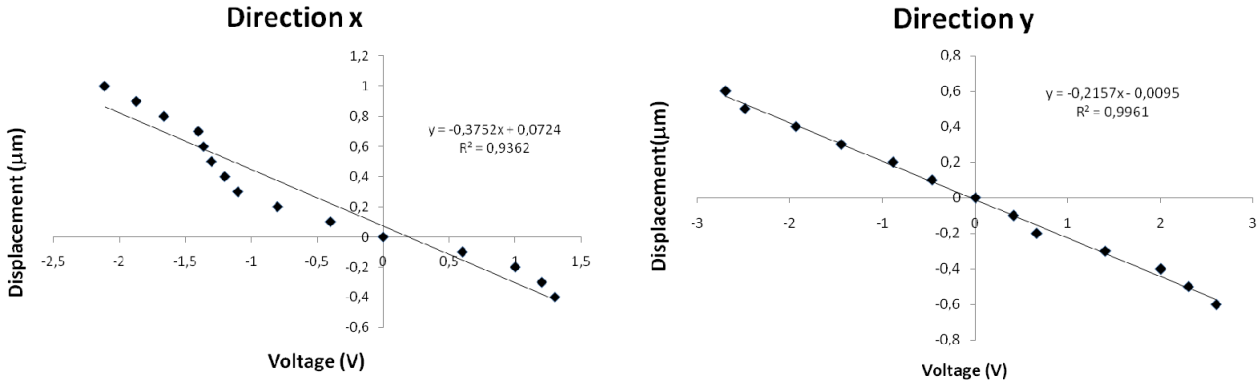

Fig 2. The x-y signal calibration plots using the quadrant detector.

We first observed the behavior of the parasite alone. When not in the presence of any chemical gradient, the parasite showed erratic movement. The forces directions were distributed randomly and the maximum strength was 0.8 pN. Figure 3 shows the plot of the vector force for this situation.

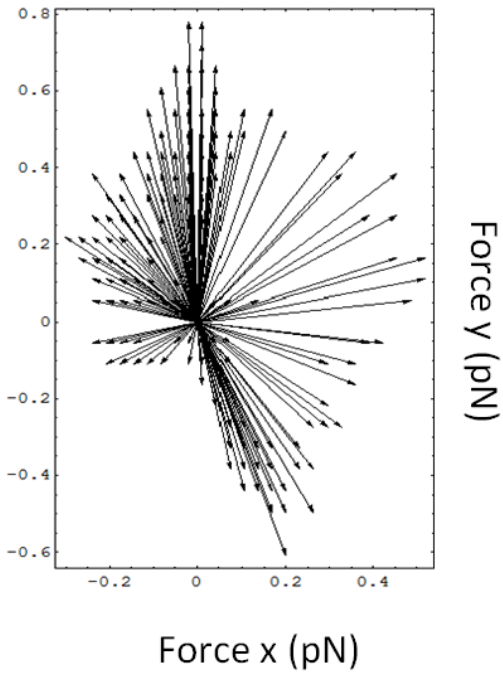

Fig 3. Plot of the vector forces for the parasite alone

By trapping and moving *T. cruzi* to the vicinities of midgut cells we observed a change in the behavior of the parasite. It projected its flagellum towards the cells as shown in figure 4. This clearly shows how the microorganism (inside the circle) can sense the presence of others cells and respond to it.

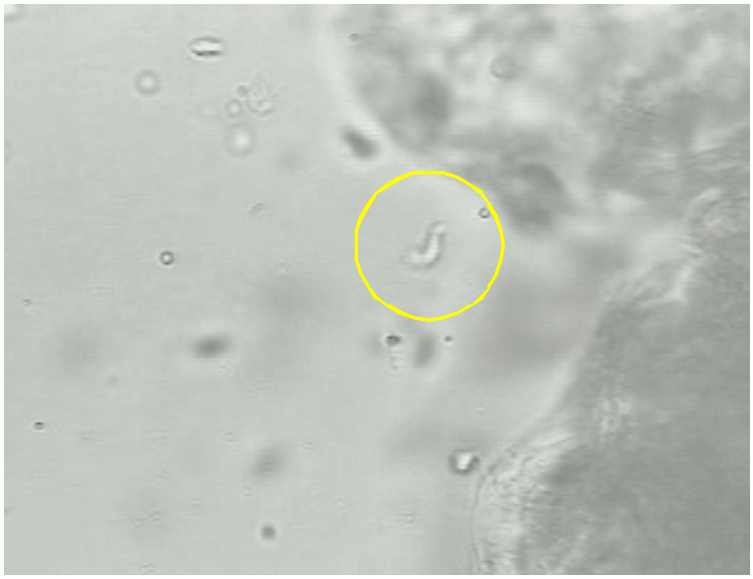

Fig 4. Optically trapped *T. cruzi* trapped and moved closer to the intestine cell.

The force vectors of the situation of figure 4 are plotted in figure 5. There are variations both in strength and direction of the forces. All the vectors are pointing towards the midgut cells. Maximum strength is 2.2 pN. This behavior contrasts with its behavior more than 50 μm away from the midgut cells where it just shows an erratic movement without any preferred direction, as shown on figure 3.

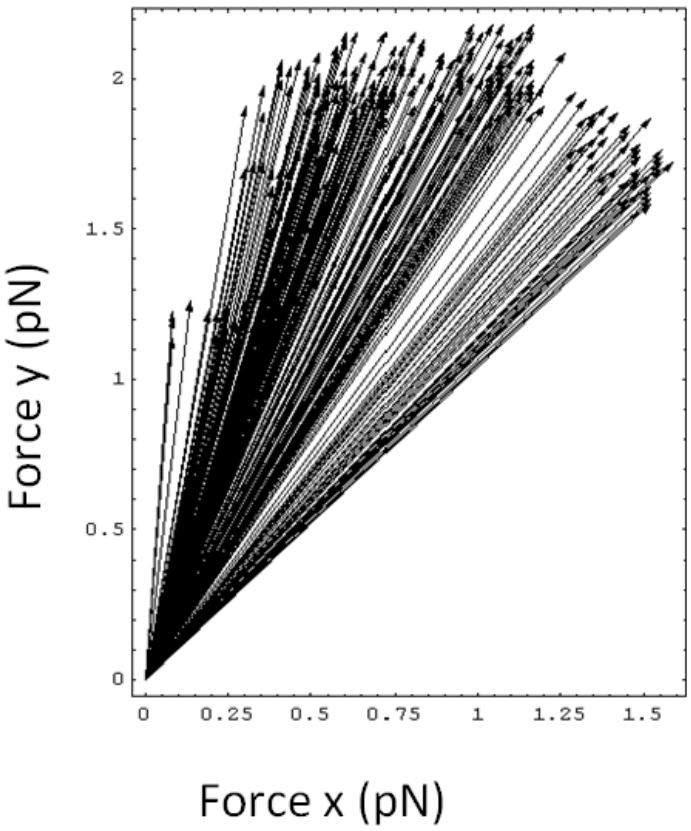

Fig. 5 Bidimensional vector force in presence of Midgut cell *of R. prolixus.*

4. CONCLUSION

In conclusion, we developed a methodology to measure in real time the bidimensional vector forces (x and y) of parasites under concentration gradients. The investigation of chemotaxis is an essential to understand the infection processes, because parasites must recognize and move towards the cells to be infected. Although we have used this system to characterize the chemotaxis processes of one specific protozoan, it could also be used to quantitatively study the taxis of any kind of microorganism under concentration gradients of several chemical substances, as well as any taxis process related to other types of gradients, such as temperature [thermotaxis], pressure [barotaxis] or osmotic pressure [osmotaxis].

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