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# Differential Tethering of Log Phase Trypanosoma brucei onto Chemically Distinct Surfaces

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# Differential Tethering of Log Phase *Trypanosoma brucei* onto Chemically Distinct Surfaces

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Our long-term objective is to understand and model the motility of *T. brucei*. Obtaining high quality images of T. brucei that allow one to differentiate between cell body movement and flagallar movement is difficult with *T. brucei* because the flagellum is attached along the cell body. Currently, our approach is to tether T. brucei onto a microscope friendly surface. The contributions to the ISIS proceedings summarize our progress to date. Specifically, we look at the adhesion density of *T. brucei* to numerous microscope friendly surfaces and at the optimum adhesion conditions for *T. brucei*.

## MODELING MOTILE CELLS REQUIRES IMAGING MOTILE CELLS

Generally speaking, two approaches are being used to model the motility of flagellated organisms. The first modeling approach begins with the fundamental components of a flagellum. The much cited, geometric clutch model proposed by Lindemann began with the fundamental components of "9+2" axoneme and was used to describe the beating of cilia and flagella [1-3]. Moreover, the model has been modified to describe multiple aspects of boar sperm motility [4]. Other researchers are taking using fundamental components to build models that describe the motility of organisms. In contrast, the second modeling approach uses both static and dynamic images of the flagellum as the initial parameters. For instance, Gueron and Liron used images of the beat pattern of a cilium of a *Paramecium* to calculate the parameters of an internal engine that depends only upon the geometry of the cilium [5]. Obviously the two approaches are complimentary and not mutually exclusive. Ultimately, any model that describes the motility of an organism must be compared with the actual motility of the organism. Most often this comparison is accomplished by imaging the organism as it moves. For these reasons, imaging motile cells is important.

## AN ORGANISM OF INTEREST: *TRYPANOSOMA BRUCEI*

In our case, we are interested in the unicellular flagellated parasite, *Trypanosoma brucei*. *T. brucei* is the causative agent for African Sleeping Sickness, which threatens

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approximately 60 million individuals in sub Saharan Africa.[6] Although the flagellum is a highly conserved structure, the attachment of the flagellum to the cell body of *T. brucei* is significantly different than other commonly modeled flagellated organisms. Unlike other flagellated cells, the flagellum of T. brucei is attached along the length of the cell body.[7] Furthermore, the flagellum of *T. brucei* is attached in a left-handed helix as it extends from the posterior to the anterior of the cell body.[7] Parallel to the axoneme and the cell body is a highly organized lattice-like structure called the paraflagallar rod. The axoneme is attached to the paraflagallar rod through the outer doublets four through seven. In turn, the paraflagallar rod and axomeme structure is attached to the cell body through the flagallar adhesion zone.

The unique architecture of *T. brucei* has several consequences. Since the flagellum is attached in a helical pattern to the cell body, the flagellum naturally moves in a three dimensional pattern. [7, 8] Furthermore, *T. brucei* uses it's flagellum to pull itself through a medium; whereas other commonly modeled flagellated organisms use their flagellum to push themselves through a medium.[8] Finally, distinguishing between movement and cell body movement is more difficult with *T. brucei* since the flagellum is attached along the length of the cell body.

The unique architecture of *T. brucei* requires novel approaches to understanding and modeling its motility, which in turn requires new imaging strategies. Currently, our strategy is to tether *T. brucei* onto microscope friendly surfaces. In these conference proceedings, we communicate the optimum conditions for immobilizing log phase *T. brucei* onto several different chemically distinct chemical surfaces. Furthermore, we compare the adhesion density of *T. brucei* on several chemically distinct chemical surfaces.

## METHODOLOGY

All chemicals were purchased from Sigma-Aldrich unless otherwise stated. Two of the four surfaces are commercially available. (Sigma-Aldrich sells the Silane Prep microscope slides while Electron Microscopy Sciences sells the poly-L-lysine microscope slides.) The other two surfaces, Sigma-cote and acid washed, are simple to prepare. For the Sigma-cote, a 15-minute incubation in fresh Sigma-Cote, followed by a thorough rinsing, produces slides with consistent hydrophobicity (as measured by contact angle meter). The acid washed slides are prepared by incubating slides in chromic acid for 24 hours followed by ample rinsing.

Briefly, tethering is accomplished by first determining the concentration (and hence growth phase) of *T. brucei* using a hemocytometer. The appropriate volume of log phase cells is centrifuged to a pellet, rinsed with PBS, and re-suspended in PBS so that the concentration of cells is $1* 10^7$ cells/mL. Fifteen microliters of the $1* 10^7$ cells/mL was applied to the various microscope slides.

An Olympus BX50 fluorescent, DIC-equipped microscope equipped with SPOT software was used to image the tethered cells. Cell counts were obtained at a 400X magnification; the approximate imaging size was about 300 microns by 300 microns. Live and dead cells were both counted.

# RESULTS

## Characterization of Microscope Surfaces

The four microscope surfaces were characterized using a contact angle meter. Table 1 details the results. Briefly, chromic acid washed slides were used as a control because of the ease of their preparation. As one would expect, water placed onto a chromic acid washed slide did not bead up. Poly-L-Lysine slides are often used to mimic cell / protein interactions. Hypothetically the surface contains charged $-NH_3^+$ moieties along with methylene ($CH_2$) units. Silane prep slides are more hydrobyobic, but still contain the ionizable $-NH_2$ group. Finally, Sigma-cote treated slides are very non-polar, as indicated by the contact angle measurment. Sigma-cote is a mixture of short chain chlorosilanealkanes and is often used to render glass surfaces hydrophobic.

**TABLE 1) Contact Angle, which serves as a measure of hydrophobicity, for 4 different chemical surfaces.**

| Chemical Treatment | Contact Angle |
|---|---|
| Chromic Acid Washed | $<5^0$ |
| Poly-L-Lysine | $48^0$ |
| Silane Prep | $68^0$ |
| Sigma-Cote ™ | $84^0$ |

## Optimum Incubation and Temperature Conditions for Tethering

Figure 1 shows the results of incubation time and temperature on tethered cell density for Poly-L-lysine slides. Incubation time refers to how long the cells were allowed to sit on the microscope slide prior to viewing. Temperature does not play a significant role in the tethering density. However, it was observed that at elevated temperatures ($26.2^oC$) be tethered cells were most likely to be dead. However, the living cells were more motile than at lower temperatures. Incubation time does play a role in how many cells are tethered to a surface. For simplicity, we choose ten minutes as the immobilization time. Similar results were obtained for chromic acid slides, Sigma-cote slides, and silane prep slides (data not shown).

## Tethering Density on Different Surfaces

The different surfaces tethered the cells with a different density and observed characteristics. The chromic acid washed slides adhered the most cells, with 26 cells per field. In general, the cells tethered on the chromic acid had a variety of tethering orientations (flagellum tethered, cell body tethered, tip of flagellum and posterior end of cell body tethered, etc…). The silane slides also demonstrated no preference for tethering orientation, and 23 cells per field were immobilized. The Sigma coated slides and the poly-L-lysine adhered approximately 15 cells per field. Interestingly, the cells tethered to the Sigma-coated slides were able to crawl along the surface.

In future work, we plan to quantify the observed orientations of cells and compare log and stationary phase cells tethering density.

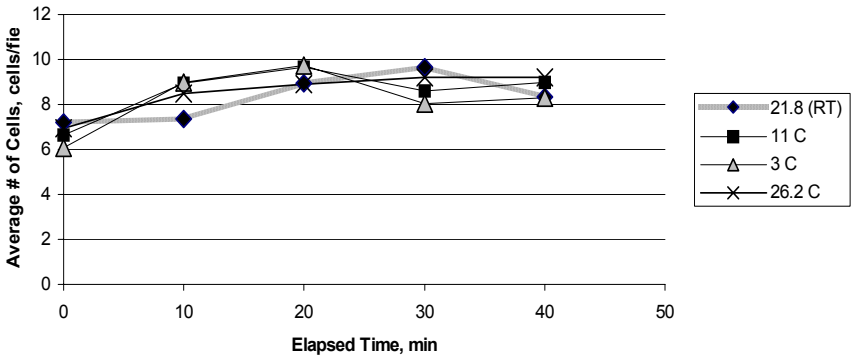


**Figure 1:** Number of cells tethered onto Poly-L-Lysine slides at four temperatures and with varying incubation times. The number of cells immobilized does not vary greatly with temperature; however, above room temperature, the cells were more likely to be destroyed although the living cells did exhibit more motility. Increasing incubation time does increase the number of cells immobilized.

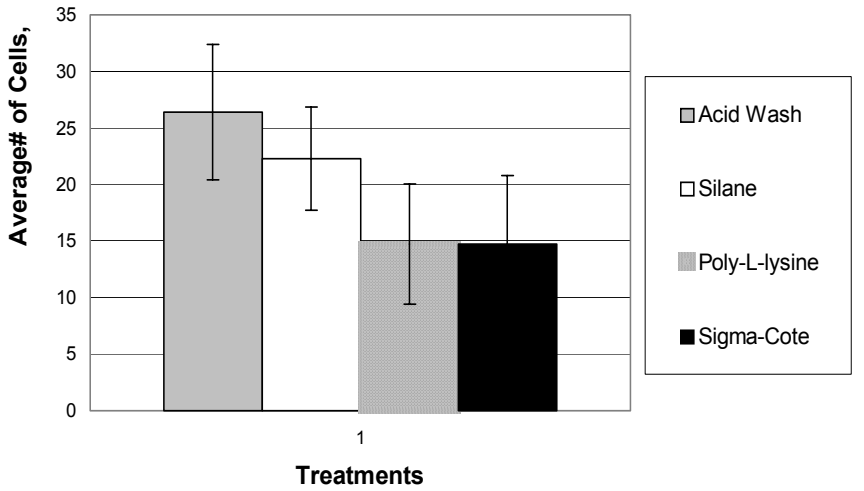


**Figure 2:** Number of cells tethered onto four different chemical surfaces. Various slide treatments yield a different number of tethered cells. Chromic acid washed slides (acid washed) adhere the most cells per field then silane treated slides. Finally, poly-l-lysine and sigma-cote slides adhere about the same number of cells.

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## REFERENCES

1. Lindemann CB. A geometric Clutch hypothesis to explain oscillations of the axoneme of cilia and flagella. J Theor Biol 168:275-189, 1994.
2. Lindemann CB. A model of ciliary functioning which uses the forces transverse to the axoneme as the regulator of dyenine activation. Cell Motil Cytoskeleton 29:141-154, 1994.
3. Lindemann CB, Kanous KS. 1995. "Geometric Clutch" hypothesis of axonemal function: Hey issues and testable Predictions. Cell Motil Cytoskeletron 31:1-8, 1995.
4. Schmitz KS, Holcomb-Wygle DL, Oberki DJ, Lindemann CB. Measurement of the force producted by and intact bull sperm flagellum in isometric arrest and estimation of the dynein stall force. Biophysics Journal 79:468-478, 2000.
5. Gueron, S. and Liron, N. Simulations of three-dimensional ciliary beats and cilia interactions. Biophys. J. 65, 499-507. 1993.
6. World Health Organization, "Tropical disease, including Pan African tsetse and trypanosomiasis eradication campaign" Report by the Seretriat. April 14, 2003. Available at http://www.who.int/gb/ebwha/pdf_files/WHA56/ea569.pdf.
7. Hill, Kent, Biology and Mechanism of Trypansome Cell Motility. Eukaryotic Cell, Vol. 2, No. 2 p[ages 200-208, April 2004.
8. Fry, D.; Hutchings, N.; Ludu, A. Dynamics of Immobilized Flagella. Los Alamos National Archives, Identifier oai:arXiv.org:physics/0309026 (20040731). September 2003.