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ORIGINAL ARTICLE

Trichomonas vaginalis multiplication in a new light – binary fission, cytokinesis or else?

Piotr Kochan[1], Agata Pietrzyk[2], Barbara Papir[3], Małgorzata Bulanda[4]

Introduction

Trichomonas vaginalis is the most common parasitic sexually transmitted disease (STD) agent. With 143 million persons infected, WHO estimates it to be the 3rd most common global STD pathogen after herpes (500 million infected), human papilloma virus (290 million), coming just before chlamydial (131 million) sexually transmitted infections (STI) and by far exceeding gonorrhoea (78 million), HIV (36.7 million) or syphilis (5.6 million) [1, 2]. Historically, trichomoniasis symptoms were fulminant in females with the males being the ones with no symptoms, but today even women may have scarcely symptomatic or asymptomatic course. In the US, most women (85%) diagnosed with trichomoniasis reported no symptoms [3]. The symptoms of STI may develop after a few days (usually from 4 days to 3 weeks), especially in women, and may include vaginitis with a purulent or frothy greenish discharge, sometimes accompanied by vulvar and cervical irritation and lesions, lower abdominal pain, dyspareunia and/or symptoms of a urinary tract infection. In males, if symptoms do occur, it may be a typical clinical picture of urethritis, epididymitis or prostatitis. According to most parasitic resources and books, incl. CDC's DPDx, *Trichomonas vaginalis* is said to divide by binary fission [4]. Usually binary fission refers to prokaryotes but certain protozoa may also use of this process. For eukaryotes, the process of cytokinesis is described as more valid. Malik *et al.* demonstrated that *T. vaginalis* has orthologs of 27 of 29 meiotic genes that include eight of nine genes which encode meiosis-specific proteins in model organisms [5]. Although authors of that article state that meiosis has not been observed in *T. vaginalis*, their findings suggest it is either currently sexual or a recent asexual, consistent with observed, albeit unusual, sexual cycles in their distant parabasalid relatives, the hypermastigotes [5]. Furthermore they agree that the parasite may be equipped to perform meiotic recombination or similar parasexual process by using its meiotic gene homologs [5]. Since many studies nowadays focus on the genotyping or molecular aspects of given pathogens, we went in a different direction and revised the multiplication of *Trichomonas vaginalis* in the light of new studies, using a classical observational approach. The results of these observations are presented in a 15-minute compilation of microscopic recordings with labels and subtitles.

Materials and Methods

Trichomonas vaginalis originating from the collection of the Laboratory of Parasitology at the Chair of Microbiology, Jagiellonian University Medical College, was cultured in test tubes with Diamond's medium at 37ºC for 24h. We were looking for actively dividing cells, which is more likely to be observed in a relatively fresh medium. Standard as well as concavity slides were selected for observations using a standard light microscope with objectives from 10-40× magnifying power. After incubation, a drop of culture medium containing the protozoa was placed on the standard microscopic slide and covered with a coverslip. After few initial observations and preliminary recordings, it was noted that such approach shortens the possible recording time, owing to faster drying and shorter lifespan of the cells, so instead the concavity microscopic slide was deemed appropriate. The slide has a tiny oval well allowing the medium to stay fluid for a longer time. The preliminary concerns regarding the focusing problems in a thicker specimen were alleviated and corrected with the automatic camera zoom setting and the microscopic micro knob, if needed. A special arm for Nikon A10 camera was attached to the ocular of the microscope and the image was set and recorded. The highest resolution offered by the camera for film recording was used, i.e. 720 HD. The image was viewed using the 10, 20 and 40× objective lenses. The authors made full use of the optical (5×) and digital zoom, so the magnifications in the film do not reflect microscope objective and ocular powers.

Film and Figure

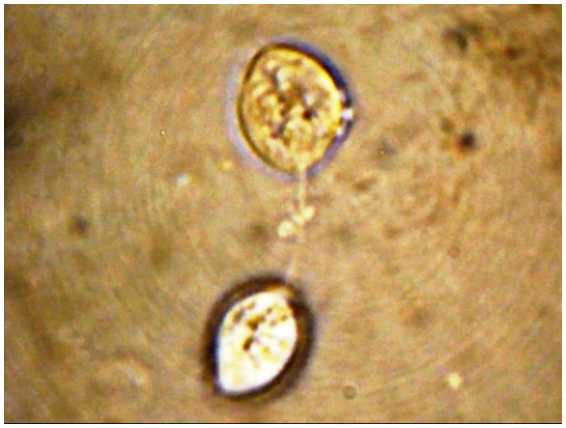

Figure 1. Still shot from the video showing a dividing trophozoite (*Trichomonas vaginalis*) bound together, in a 24h Diamond's medium (10-40× magnification with digital zoom). Please see the full film on WJOMI YouTube channel here: https://youtu.be/cyAenYJ76VU

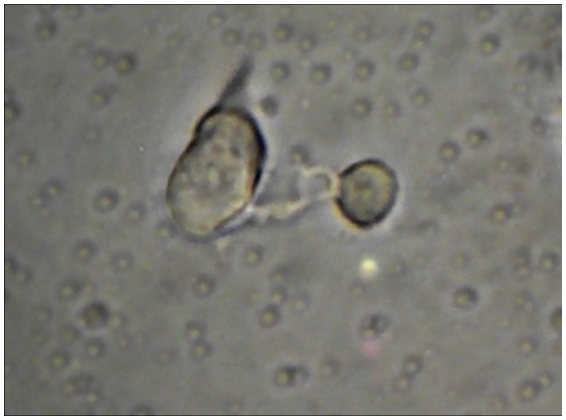

Figure 2. Photograph showing the parent trophozoite and daughter cell, still bound by a structure resembling the human umbilical cord, which does not seem to be always related to the axostyle – we called it the "Kochan cord" (24h Diamond's medium, 40× magnification with digital zoom).

Conclusions

Our suspicions on *T. vaginalis* multiplication were initially raised by the fact, that medical students were asking us during classes why do live *T. vaginalis* cells under the microscope have different sizes, despite them dividing by binary fission, which should in fact warrant same or similar sizes. It was a delicate matter, since all students' textbooks and the lifecycle chart we recommend for student reviews, which may be found on DPDx website, the images there show beautiful oval trophozoites dividing evenly into two cells of the same size, along their long axis (Figure 3). It was difficult to explain the size differences in real life.

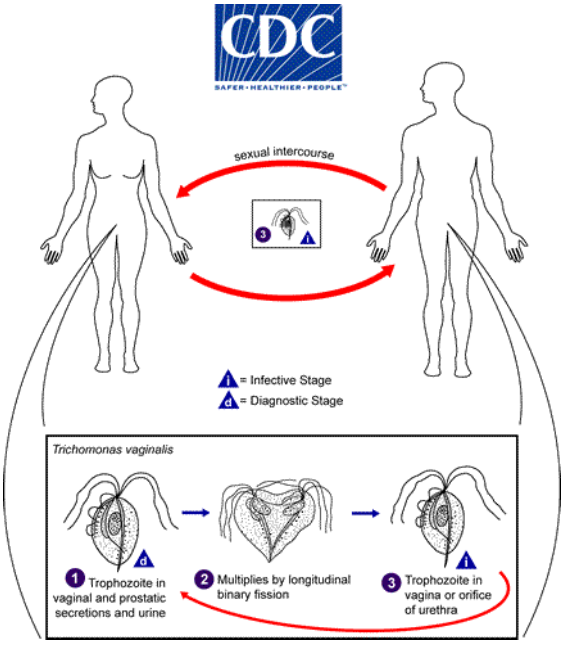

Figure 3. Life cycle of *Trichomonas vaginalis* from DPDx website, which is developed and maintained by CDC's Division of Parasitic Diseases and Malaria (DPDM) [4].

The film without any doubt explains these discrepancies and major differences in cell size. Usually the offspring cell is smaller, sometimes significantly, but may grow in size similar to the parent during the division process. We consider this to be the first step in understanding the *T. vaginalis* cell division, using a classical microscopic observational study, complementary to findings of Malik *et al.* [5] Considering our *in vitro* observations, the division of this important sexually transmitted parasite shown in medical handbooks or web sources is shown in shorthand , or even, placing more emphasis on this statement, it's portrayed erroneously.

We noted a few stages each time, repeated in all dividing cells, which may in fact more closely resemble cytokinesis. In the film featured in this article (Figure 1), the multiplication process is as follows:

1. Trophozoite at first forms a tumorous bleb extending from its surface;
2. The bleb grows in size more and more, as if a balloon was inflated with helium, resembling a small trophozoite;
3. A kind of an umbilical cord is formed between the parent and daughter cell, and from our observations, although common, it's not always via the axostyle – we called it the "Kochan cord" (pronounced "kohancord", which is also close to the pronounciation of "anchor"). Please see the following time codes in Figure 1 in the film: "Kochan cord" best visible on 3:36 and 0:36 and Figure 2;

4. Usually another trophozoite comes near the multiplying one. We named it the "midwife" trophozoite;
5. The “midwife” trophozoite in many cases stays close to the dividing cells and seems to help in detachment of the offspring trophozoite. This is not a rule, though. Please see the following time codes in Figure 1 in the film: progeny cells liberated with the help of "midwives" on: 7:15 and 14:55;
6. The process is not so easy, sometimes both or just the parent trophozoite dies;
7. The offspring trophozoite, using its flagella, rotates violently, turns around its own axis vigorously and moves energetically to liberate itself from the parent cell;
8. The whole process usually takes several to even 15 minutes (esp. in drying specimens).

It’s hard to say whether the fifth flagellum plays a role during division or whether it has some sort of rudder/sensory function, directing the movement of the cell.

The limitation of this short study, as with any *in vitro* observations, is the fact that *in vivo* phenomena may always bear the risk of an alternative pattern.

To sum up, the division of *Trichomonas vaginalis* cells, that we were able to film is not corresponding to descriptions in the literature. We will continue working on this subject and surely, we will be able to answer more questions coming up ahead.

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Conflict of interest: P.K. is WJOMI’s Editor-in-Chief.

Authors’ affiliations:
[1] Chair of Microbiology, Department of Bacteriology, Microbial Ecology and Parasitology, Jagiellonian University Medical College in Cracow, Poland
[2] Head of the Parasitology Lab, Department of Bacteriology, Microbial Ecology and Parasitology, Jagiellonian University Medical College in Cracow, Poland
[3] Parasitology Lab, Department of Bacteriology, Microbial Ecology and Parasitology, Jagiellonian University Medical College in Cracow, Poland
[4] Head of Chair of Microbiology, Jagiellonian University Medical College in Cracow, Poland

Corresponding author:
Piotr Kochan, M.D., Ph.D.
Chair of Microbiology
Jagiellonian University Medical College
18 Czysta Str., 31-121 Cracow, Poland
e-mail: pkochan@cm-uj.krakow.pl

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