Some Difficulties in Research into Cell Motile Activity under Isotropic Conditions*

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Movement of Dictyostelium discoideum amoebae under isotropic and anisotropic conditions was recorded and analysed with computer-aided methods and the results are presented in various manners as described in the subject literature. Cell movement under isotropic conditions showed great diversity. Some cells moved almost in a straight path whereas others in close proximity turned around with little net translocation. When cell movement under isotropic conditions was observed, no direct correlation was found between the total length of cell trajectories and the length of final displacements of the cells. It was necessary to present the results in the form of histograms, circular diagrams of cell trajectories or in scatter correlation diagrams showing the motile behaviour of many individual cells. These methods of presentation are more informative than methods which present only average values, the “representative” behaviour of single cells, or start and end points of cell tracks. The latter methods can only illustrate but do not document the results of experiments. The use of statistical methods appears necessary in cases when it is difficult to monitor the same cells before and during experimental treatment. However, when cell movement under anisotropic conditions becomes oriented and ordered as during tactic cell movements, then the diversity in cell behaviour decreases and methods based on estimation of starting and end points of cell positions appear more credible.

Key words: Dictyostelium discoideum, cell movement, results presentation.

Abbreviations used:
D. discoideum – Dictyostelium discoideum,
BSS – basal or basic salt solution,
FA – folic acid,
CME – coefficient of movement efficiency

Cell movement in metazoan organisms plays an important role in embryogenesis, regeneration, immune responses and spread of metastases. In Protista or slime moulds it is necessary during foraging or aggregation and formation of pseudoplasmodia and fruiting bodies (ANTON et al. 1996; ARKOWITZ 1999; ETTENSOHN 1999; FORBES & LEHMANN 1999; FRIEDL et al. 2001; KESSIN 2001; MOLYNEAUX & WYLIE 2004; TRINKAUS 1973). A variety of methods have been developed to study and register cell motile activity and the results of these studies are presented in many various ways in the subject literature. In particular, when experiments concern cell populations rather than single cells, this diversity of methods causes difficulties when results of experiments carried out in different laboratories are compared.

In the presented research the results of experiments carried out on Dictyostelium discoideum amoebae moving under isotropic and anisotropic conditions are compared and presented in a few diverse forms found in the subject literature. The results show that when the research concerns the movement of cells under isotropic conditions, it is insufficient to show chosen “typical” examples of cell behaviour or to determine only starting and

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end positions of observed cells. The recording of cell trajectories and presentation of results in the form of circular diagrams, histograms or scatter correlation diagrams (dot-plots), are necessary. These problems appeared less significant and a correlation between final translocation of cells and the length of cell trajectories occurred when more oriented and ordered cell translocations during chemotaxis were investigated.

Material and Methods

Cell Culture

Dictyostelium discoideum strain AX-2 (ATCC24397) was cultured as described previously (KOROHODA et al. 2002; SROKA et al. 2002) in medium containing: 14.3g/l meat peptic pepton (Oxoid Ltd, England), 7.15g/l yeast extract (BioMerieux, France), 18g/l maltose (Sigma St. Louis, MO), 1.28g/l Na2HPO4 x 1 2H 2O, 0.48g/l KH2PO4, adjusted to pH 7.4. The cells were grown in a suspension at 20 oC. Cells at the exponential growth stage were used in experiments. They were washed three times with Chalkley’s solution (14mM NaCl, 0.27mM KCl, 0.5mM CaCl2, 0.5mM MgCl2) (HABEREY & STOCKEM 1971; KOROHODA & STOCKEM 1975) or Basic Salt Solution (20mM KCl, 2.5mM MgCl2, 20mM KH2PO4) (WESSELS et al. 2004), resuspended in an appropriate medium (Chalkley’s solution or BSS) and plated in Petri dishes.

Cellular motility under isotropic conditions

Motility of D. discoideum cells was observed in Chalkley’s solution or in Basic Salt Solution (BSS). For the experiment with D. discoideum amoebas after starvation, cells were incubated for 6h at 20oC (KOROHODA et al. 2002; VAN DUIJN & VAN HAASTERT 1992) in BSS. The cell suspension was washed three times, shaken on a rotary shaker and resuspended in Chalkley’s solution or BSS. The cells were observed after being spread in a Petri dish and their motility was recorded.

Cellular motility in a developing concentration gradient of chemoattractant

Motility of the cells migrating in a developing concentration gradient of folic acid (FA), which is a chemoattractant for D. discoideum, was observed in a chemotactic pocket-like chamber (KOROHODA et al. 1997). The chambers were made of microscopic cover-glasses of the following dimensions: the bottom glass 35 x 60 mm, the lid 20 x 20 mm, the sides 20 x 5 mm. Each chamber has a U-shaped form, closed on three sides and the one open possesses inner dimensions of 5 x 5 x 0.2mm (for more details see: KOROHODA et al. 1997; KOROHODA et al. 2002; SROKA et al. 2004). During the experiment the chamber was placed in an external solution containing FA (0.5mM) in Chalkley’s solution. Cells were placed in the chamber, allowed to attach to the glass surface and after 30 minutes were recorded for 20 minutes in the developing concentration gradient of FA.

Recording of cellular motility

Cell migration was observed with an inverted Hund Wilovert S microscope. Tracks of individual amoebae were generated as described previously (KOROHODA et al. 1997; KOROHODA & MADEJA 1997; SROKA et al. 2004). The images of migrating cells were recorded and analysed by computer-aided methods. Images were acquired every 20s for 20 minutes. Tracks of migration were constructed by determination of the cell centroid position for each frame; the whole trajectories were composed of 60 subsequent centroid displacements. The cell trajectories were presented in circular diagrams with the starting point of each trajectory situated in the diagram centre (GRULER & NUCITELLI 1991; KOROHODA et al. 1997; KOROHODA & MADEJA 1997). Histograms and correlation diagrams of trajectories and total length of cell displacement were also prepared.

Analyses of cellular motility

The following parameters characterising cell locomotion were computed for each cell or cell population:

– the total length of the cell trajectory,
– the total length of cell displacement – the distance between the first and the last points of the cell track,
– coefficient of movement efficiency (CME) – the ratio of cell displacement to cell trajectory length; the CME is 1 for cells moving persistently along a straight line and 0 for random movement (FRIEDL et al. 1993; KOROHODA et al. 1997; SROKA et al. 2004).

GAIL and BOONE (1970) introduced the phenomenology of a “persistent random walk” to describe the random locomotion of cells. In experiments, the mean square displacement against time and the motility constant (the augmented diffusion constant) are estimated and used to characterise cell locomotion (DUNN & BROWN 1987; GAIL 1973, GRULER 1984; PADDOCK & DUNN 1986). The augmented diffusion constant D* was com-
puted from the plot of the mean square displacements against time from the equation:

\[ \langle \tau^2 \rangle = 4D^*(t-t^*(1-\exp(-t/t^*))) \]

where \( D^*, t^* \) are constants; \( T \) – the length of cell displacement from the starting point to the subsequent position; \( t \) – time (GAIL 1973, GAIL & BOONE 1970; GAIL & BOONE 1971).

Statistical significance was determined by Student’s \( t \)-test or a nonparametric Mann-Whitney test with \( P<0.05 \) considered significant.

**Results**

In Figure 1A a microscopic field is shown in which two perimeter and centroid tracks of selected *D. discoideum* are marked; the amoebae were moving under isotropic conditions for 30 minutes at room temperature. In Figure 1B the changes in the shape of these cells are extracted and shown, and in Figure 1C the difference in the total length of the cell trajectory and the total length of the cell final displacement are displayed.

Fig. 1. The trajectories (A) and contours (B) of two *D. discoideum* amoeboid cells (a’ and b’) migrating under isotropic conditions in Chalkley’s solution. The trajectories of each cell in (A) were constructed from 60 successive positions recorded at 20 s intervals. The contours of cell migration shown in (B) were taken every 80s for 20 minutes. C: Diagram to illustrate the different trajectory lengths and cell displacement by the two cells depicted in A and B.

Fig. 2. A, B: The trajectories of *D. discoideum* cells (n=100) migrating under isotropic conditions in BSS solution (A) and cells after 6h of starvation (B). In all diagrams the initial point for each trajectory was placed at the centre of the circle. Each trajectory was constructed from 60 successive positions of the cell centroid recorded at 20 s intervals. C, D: Scatter correlation diagrams of total trajectory and displacement of *D. discoideum* cells migrating under isotropic conditions in BSS solution (C) and in BSS solution after 6h of starvation (D) n=100.
Disparate shapes of cell trajectories and different changes in cell shape of two single cells from the same culture conditions can be seen. Whereas the cell marked “a’” moves along almost a straight path, the cell marked “b’” often turns and its resulting net translocation is very small.

To further characterise the cell motile activity under isotropic conditions, the trajectories of many single cells were determined and statistically evaluated. In Figure 2A and Figure 2B the trajectories of *D. discoideum* amoebae migrating in BSS solution under isotropic conditions for fed amoebae, i.e. newly harvested from the growth medium (A), and amoebae starved for 6 h in pure mineral salt solution (BSS) (B), are displayed in the circular diagrams (FARBOUD et al. 2000; KOROHODA et al. 1997; KOROHODA et al. 2002; NUCCITELLI et al. 1993; WANG et al. 2000).

In Figure 2C and Figure 2D the distribution of values of the length of cell trajectories and the length of final cell displacements of 100 single cells are displayed in corresponding scatter correlation diagrams, such as those commonly used to display the results from flow cytometry measurements and suggested by FRIEDL et al. (1993) for use in the analysis of cell movement. The absence of a correlation between these two parameters characteristic for cell trajectories in the case of fed amoebae moving under isotropic conditions is visible, and the value of the correlation coefficient is only 0.26. Whereas in Figure 1 great differences in cell motile behaviour and cell turning between cells from the same culture conditions are shown, results of measurements of cell displacements and lengths of cell trajectories shown in Figure 2C and Figure 2D demonstrate that when the cells move under isotropic conditions all intermediate types of turning behaviour can occur in one population. Long trajectories and short displacements correspond to cells which often changed the direction of locomotion and long trajectories and long displacements characterised cells which moved almost straight (cf. Fig. 2D).

In the case of starved amoebae (Fig. 2D), there is a slightly better correlation between the total length of cell trajectories and the total length of cell displacement (the correlation coefficient is 0.32), and in the corresponding scatter diagram a subpopulation of cells which show more straight trajectories appears. The corresponding histograms for fed and starved amoebae are shown in Figure 3.

The effect of starvation upon the motile behaviour of amoebae is further confirmed by random walk cell movement analysis. The corresponding mean square displacements are shown in Figure 4.

Subsequent experiments concerned the effects of univalent cations present in the solutions used in experiments on *D. discoideum* amoebae and cell movement orientation in the chemotactic concentration gradient of folic acid (FA) upon the cell motile behaviour. The BSS solution used by some investigators on *D. discoideum* amoebae contains 20 mM KCl and 20 mM KH2PO4 but no sodium, others often used Na/K phosphate buffer as a main component of the mineral salt solution used for research on cell motility (usually at 16 mM to 40 mM phosphate buffer concentration, pH 6.0 to 6.4) (SOLL 1987; SONNEMANN et al. 1997; WESSELS et al. 1996), whereas Chalkley’s solution is most commonly used in research on other amoebae and species of Protista and contains 14 mM NaCl and only
0.27 mM KCl as salts with monovalent cations (HABEREY & STOCKEM 1971; KOROHODA & STOCKEM 1975). Therefore, the movement of *D. discoideum* amoebae freshly harvested from the growth medium in these two different solutions was compared with cell movement in a chemotactic concentration gradient of folic acid (FA) in Chalkley’s solution. The results are shown in the form of circular diagrams and scatter correlation diagrams in Figure 5A-5F. There is no significant difference in cell motile behaviour when fed amoebae move under isotropic conditions in the two solutions examined (Fig. 1A, 1B, and 1D, 1E), but when cell movement becomes oriented and

![Circular diagrams](image1)

![Scatter diagrams](image2)

**Fig. 5:** A-C: The trajectories of *D. discoideum* cells migrating under isotropic conditions. In circular diagrams the initial point for each trajectory was placed at the centre of the circle. Each trajectory was constructed from 60 successive positions of the cell centroid recorded at 20 s intervals. The trajectories of *D. discoideum* cells migrating (A) in Chalkley’s solution (n=100), (B) in BSS solution (n=100); (C) *D. discoideum* migrating within the positively developing gradient of folic acid (FA) in Chalkley’s solution (solution within the chamber at the beginning of the experiment without FA and the external solution containing 0.5mM FA) (n=50). D-F: Scatter diagrams showing correlation of the total length of cell trajectories and the total length of cell displacement of *D. discoideum* cells migrating in Chalkley’s solution (D) (n=100), BSS solution (E) (n=100) and cells migrating within the positively developing gradient of FA (F) (n=50). Coefficient of correlation equals 0.26 for cells migrating in Chalkley’s solution, -0.11 for cells in BSS and 0.95 for cells in the positively developing gradient of FA.
cells progress in the gradient of FA their movement drastically changes. The cells migrate along almost straight tracks and the correlation coefficient between the total length of their displacement and the total length of their trajectories reaches the value of 0.95.

The same difference in cell behaviour under isotropic and anisotropic conditions is clearly visible when the results are displayed in the form of histograms (Fig. 6A-6C) (cf. also Table 1).

Discussion

The results presented in this communication show that when cell movement under isotropic conditions has to be documented it is insufficient to present a few selected tracks or one chosen series of cell tracks or cell shape changes as “typical” or “representative” (ROYAL et al. 1997). The differences in motile behaviour of single cells in the same isotropic experimental conditions often appear to be much greater than the differences which are assumed to be caused by the studied factor introduced in an experiment. To determine whether the change in final cell translocation is caused by a change in the speed of cell movement or a change in the shape of the cell trajectory, i.e. cell turning behaviour, it is necessary to determine the tracks of moving cells (KELLER & ZIMMERMANN 1985; KOROHODA et al. 1997; KOROHODA et al. 2000; SOLL 1995; TRANQUILLO & ALT 1990; VAN DUIJN & VAN HAASTERT 1992). The diverse behaviour of single cells in proximity under isotropic conditions renders even precise recording methods of cell movement, such as cell tracks or records of cell motile activity displayed as outline tracks showing the motile activity of chosen single cells, appear insufficient. Cell

<table>
<thead>
<tr>
<th>Parameters of cell movement (± SEM)</th>
<th>Isotropic conditions</th>
<th>Anisotropic conditions</th>
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<tbody>
<tr>
<td>Chalkley solution</td>
<td>BSS solution</td>
<td>FA gradient</td>
</tr>
<tr>
<td>(n=100)</td>
<td>(n=100)</td>
<td>(n=50)</td>
</tr>
<tr>
<td>Total length of cell trajectory (µm)</td>
<td>283.46±6.63</td>
<td>310.52±6.02</td>
</tr>
<tr>
<td>Total length of cell displacement (µm)</td>
<td>54.48±4.18</td>
<td>66.65±3.79</td>
</tr>
<tr>
<td>CME 1)</td>
<td>0.19±0.01</td>
<td>0.22±0.01</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.26</td>
<td>-0.11</td>
</tr>
<tr>
<td>Augmented diffusion constant D* (µm²/min) 2)</td>
<td>60.12±0.66</td>
<td>69.48±1.15</td>
</tr>
<tr>
<td>Persistence in direction with time t* (min) 2)</td>
<td>0.20±0.12</td>
<td>0.35±0.19</td>
</tr>
</tbody>
</table>

1) – Coefficient of movement efficiency – the ratio of cell displacement to cell trajectory length.
2) – The augmented diffusion constant D* and the persistence in direction with time t* were obtained by fitting a theoretical model of persistent random walk to data consisting of the mean square displacements of the cells according to the method of GAIL and BOONE (1970). The fitting function is given by:

<\text{T}^2> = 4 D*(t- t*(1-exp(-t/ t*))), where D* and t* are constants and t is time.

# – Statistical significance at P<0.05 vs. parameters of cells in Chalkley’s solution.

Fig. 6. Histograms of the total length of cell trajectories and of the total length of displacements of the _D. discoideum_ cells migrating in: A – Chalkley’s solution, B – BSS solution, C – Folic acid gradient in Chalkley’s solution. Cells were centrifuged twice, then suspended in the solution depending on the experimental conditions and planted in the plates. After attachment to the substratum, cell movement was recorded for 20 minutes and the positions of cell centroids were determined at 20 s time intervals (n=50).
outline tracks had been for years determined on the basis of frame-by-frame analysis of cine-films (cf. for example BOVEE 1964) but at present they are produced with computer-aided methods (SOLL 1995; STITES et al. 1998). They show the motile activity of cells which slowly displace but actively change shape or move around often changing the direction of locomotion. Nevertheless, when very diverse behaviour of individual cells is observed as in Figure 1 and Figure 2C, 2D, it appears necessary to present data which include a quantitative description of the motile behaviour of a significant number of randomly selected cells (50 to 200 or more). There are several ways to elaborate the results of such measurements and some of the most commonly used ones are compared in this communication.

When cells move in isotropic conditions, single cells even in close proximity can behave differently, some moving along straight trajectories when their neighbours turn around with almost no displacement. As shown in Figure 2C-2D, when statistical methods are applied a lack of correlation occurs between the length of cell trajectories (which show cell movement activity) and the final cell displacement if cell movement under isotropic conditions is analysed. Thus it is necessary to record movement of many single cells and to determine the trajectories of cell displacements. The trajectories of moving cells can be displayed in the form of circular diagrams showing simultaneously the shape and orientation of cell tracks (cf. Figs 2 and 5). Histograms of dispersion and frequency of results of measurement of the length of cell trajectories and final cell displacements give information on the heterogeneity of the cell population studied (DJAMGOZ et al. 2001). The heterogeneity of the cell population and a correlation between various measured parameters can be easily shown with scatter correlation diagrams. Such diagrams are commonly used to display results from flow cytometry, but can be valuable for presentation of results of measurements concerning cell motility or its modifications under experimental conditions (FRIEDL et al. 1993). In research concerning tissue cells, often only starting and end cell positions are recorded. This corresponds to determination of final cell translocation. A statistically significant correlation between cell displacement and length of their trajectories appears when movement is oriented during chemotaxis (cf. Fig. 5F). This seems to justify the use of simplified methods based on the estimation of starting and final points of positions during an experiment when cells displace along straight tracks (not only during chemotaxis, but also during galvanotaxis or when cell movement is oriented by contact guidance on anisotropic surfaces) (FARBOUD et al. 2000; WANG et al. 2000; WOJCIAK-STOTHARD et al. 1995).

Analysis of cell trajectories is often carried out with the methods based on random walk theory (KOROHODA et al. 1997; KOROHODA et al. 2002; KOROHODA & MADEJA 1997). These methods, introduced to cell movement analysis by GAIL and BOONE (GAIL 1973; GAIL & BOONE 1970; GAIL & BOONE 1971), DUNN and BROWN (1987) and others (PETERSON & NOBLE 1972), later popularised by GRULER (1984), when applied to our results lead to consistent conclusions with those derived from presentation of results in the form of histograms and scatter correlation diagrams. For example, all three methods confirmed quantitatively (cf. Figs 2 and 4) earlier observations (ALEXANDER et al. 1992; WEBER et al. 1995) that starved D. discoideum amoebae move with less tendency to change the direction of translocation than well fed amoebae. Nevertheless, the random walk analysis can be used only for uniform cell populations and can not show heterogeneity in cell motile behaviour (cf. DJAMGOZ et al. 2001). The presentation of results with standard deviations and/or standard errors without documentation of the measured values’ distribution appears insufficient to illustrate and document homogeneity or heterogeneity of the cell population studied.

In summary, the results presented and discussed in this report lead to the following conclusions:

(i) When cells move under isotropic conditions a broad range of cell turning behaviour is observed. Under such conditions there is no correlation between cell motile activity (length of cell trajectories) and effective translocations (length of cell final displacement);

(ii) To analyse cell motile activity under isotropic conditions it is necessary to determine single cell trajectories and not only the starting positions and final locations;

(iii) When it is difficult to monitor the same cell before and during experimental treatment, it is necessary to use statistical methods. For cells moving under isotropic conditions, presentations of single cells trajectories as “representative” can only illustrate but do not document the results of experiments;

(iv) When cells move along straight paths as during tactic movements, their behaviour is more ordered and presentations of the starting positions and the end points of cell locations appear justified;

(v) Presentation of distribution of values of measured parameters characteristic for cell movement gives more information than the average values. Histograms and scatter correlation diagrams deliver additional information on homogeneity or heterogeneity of cell behaviour in the population.
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References


