

Directed Cell Movement with Steric Exclusion

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The space-dependent density of cells is evaluated for the following situation: (i) The cells are forced to make a directed movement (ii) the space for the cellular migration is restricted. The steady state distribution density is obtained when the drift current density equals the diffusion current density. The analogy to the Boltzmann statistics is shown. In a further step the cellular volume is introduced. For this case the density distribution is described in analogy to the Fermi statistics. The necrotactic response of granulocytes is used to verify the model.

Introduction

The ability of a cell, of a microorganisms, *etc.*, to detect, and to respond to, its environment is a widely spread phenomenon in biology [1]. The directed movement of cells is of crucial importance. To name a few examples: (i) Immune system [2]: the first defense line of a mammal against invaded microorganisms are polymorphonuclear leukocytes (= granulocytes). These cells are attracted to sites of inflammation by using their chemotactic or galvanotactic capability to form cellular clusters enveloping the microorganisms. Finally, the granulocytes eliminate the microorganisms by phagocytosis. Such a situation is described by an *in vitro* experiment at the end of this article. (ii) Embryogenesis, wound healing, and tumor invasion [3]: the directed movement of cells during embryogenesis, wound healing, and tumor invasion has been well documented. In particular, there are many instances, during embryogenesis, of embryonic cells migrating individually and consistently following a precise pathway toward their final destination. To understand such phenomena the interaction between single migrating cells has to be considered. In this article the steric repulsion of cells will be discussed.

The environment of a cell is the cause of external stimuli. Consequently, different environmental conditions lead to different types of directional stimuli [2]. The extracellular guiding signal can be chemical in nature: the resulting directed movement is then called chemotaxis. The signal may

also be electrical in nature: the directed migration is then called galvanotaxis. Cells can also be guided by the physical topology around them. This type of movement is called contact guidance.

It has been found that the translational kinematics of cells like granulocytes [4, 5], monocytes [6], fibroblasts [7], neural crest cells [8], in an extracellular guiding field like an electric field or concentration gradient, can be described by two independent cellular responses: the speed and the direction of migration. In addition, it has been found that the cellular speed is adjusted by a steerer (= controller without feedback) and that the angle of the direction of migration is controlled by an automatic controller (= controller with feedback). Thus, chemotaxis, galvanotaxis, and contact guidance are responses of cells which have an automatic controller as goal-seeking system [9, 10].

We now understand a great deal how a single cell interacts with the extracellular guiding field, but the interaction between different cells has been disregarded so far. Thus the theoretical considerations hold only for dilute cellular systems where the mean distance between the cells is large compared with the size of the cell. In this article we want to show how the directed movement of concentrated cell suspension can be studied.

Space-Restricted Cell Migration

To begin with let us consider N_0 cells migrating in a closed area of the length, L , and the width, w . (A similar treatment holds for the three-dimensional space.) The field which induces a cellular drift movement is applied in the x -direction parallel to one of the sides of the rectangular area. First we shall establish the steady state probability distribution of the cell density.

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Let us consider two adjacent area elements of identical size, $w\Delta x$. The cells can migrate from one element to the other. There exist two types of flows (Fig. 1):

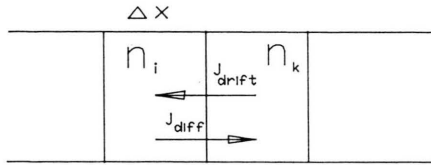


Fig. 1. Schematic representation of the flows of cells.

The migrating cells try to reach maximum uniformity. According to Fick's first law a net current of cells is expected if there exist a difference, Δn , in cell concentrations.

$$j_{\text{diff}} = -D \frac{\partial n}{\partial x}. \quad (1)$$

D is the diffusion coefficient of the cells under the used experimental conditions. The partial derivative of the density, n , as a function of x can be approximated by $\frac{\partial n}{\partial x} = \frac{\Delta n}{\Delta x}$. Δx is the mean distance between the two area elements.

The external field acts on the migrating cells in such a way that on average, the migrating cells drift parallel to the applied guiding field. The mean drift velocity can be approximated by the product of the mean track velocity, $\langle v_c \rangle$, and the polar order parameter, $\langle P_1 \rangle$ which is the average of $\cos \Phi$, where Φ is the angle between the applied field and the direction of migration. For simplicity, let us assume a constant external field so that the induced polar order parameter and the drift velocity are constant.

$$\langle v_{\text{drift}} \rangle = \langle v_c \rangle \cdot \langle P_1 \rangle. \quad (2)$$

The current density of the migrating cells induced by the external field is then

$$j_{\text{drift}} = n \cdot \langle v_{\text{drift}} \rangle = \langle v_c \rangle \cdot \langle P_1 \rangle \cdot n. \quad (3)$$

The total net current, j , as the sum of diffusion and drift current is zero in steady state.

$$j = j_{\text{diff}} + j_{\text{drift}} = \langle v_c \rangle \cdot \langle P_1 \rangle \cdot n - D \frac{\partial n(x)}{\partial x} = 0. \quad (4)$$

This differential equation can be rewritten by separating the variables. In addition, the partial

derivative can be replaced by the normal derivative since n is only a function of x at steady state.

$$\frac{dn}{n} = \frac{\langle v_c \rangle \langle P_1 \rangle}{D} dx. \quad (5)$$

Integrating the right side from $n(0)$ to $n(x)$ and the left side from 0 to x yields

$$n(x) = n(0) \cdot e^{\frac{\langle v_c \rangle \langle P_1 \rangle}{D} \cdot x}. \quad (6)$$

We obtain for the density of migrating cells a similar expression as for the gas density (at constant temperature) in the barometric formula, a special case of the Boltzmann statistics.

$$n(x) = n(0) \cdot e^{-\frac{mg}{kT} \cdot x}. \quad (7)$$

The drift velocity, $\langle v_c \rangle \langle P_1 \rangle$, in Eqn. (6) has to be replaced by the gravitational force, mg , and the diffusion coefficient by the thermal energy, kT .

In the absence of a guiding field, the polar order parameter is zero and a constant cellular density is predicted. The cells are spread uniformly over the space as expected for random walk. If, however, there is a field applied to guide the migrating cells, the polar order parameter is non zero, and the migrating cells are concentrated on one side. By means of the applied guiding field the cells have the possibility to deviate from the law of maximum uniformity.

The characteristic length of the exponential decrease is

$$l = \frac{l_0}{\langle P_1 \rangle} \quad (8)$$

$$l_0 = \frac{D}{\langle v_c \rangle}. \quad (9)$$

The characteristic length l_0 obtained for a polar order parameter of ± 1 is in the order of the cell size as we can see if we take measured values for the diffusion coefficient and the mean track velocity. For human granulocytes at 37 °C [11]: $l_0 \approx 12.5 \mu\text{m}$ with $D \approx 300 \frac{\mu\text{m}^2}{\text{min}}$ and $\langle v_c \rangle \approx 24 \frac{\mu\text{m}}{\text{min}}$.

The mean diameter of a granulocyte is approximately 15 μm .

Eqn. (6) holds only for dilute cellular systems. The interaction between different cells is not yet considered. In the next step, the steric exclusion will be introduced.

Steric Exclusion

To account for saturation of the space at places having a high cell concentration we shall invoke simple steric exclusion. Again let us consider N_0 cells migrating in a closed area of the length, L , and the width, w . The cellular flux from the area element, i , to the adjacent area element, k , is proportional to*

- first, the transition probability, $p_{i \rightarrow k}$,
- second, the cell density, n_i , in the area element, i , and
- third, the density of free spaces, $n_0 - n_k$, in the area element, k . The area, a_0 , is occupied by one cell and thus the saturation cell density, n_0 , is $\frac{1}{a_0}$.

In the previous section, this item was disregarded and consequently the previous calculation only holds for dilute systems (–for very low cell densities).

The cellular flow from i to k is

$$p_{i \rightarrow k} \cdot n_i \cdot (n_0 - n_k) \quad (10)$$

and the cellular flow from k to i is

$$p_{k \rightarrow i} \cdot n_k \cdot (n_0 - n_i). \quad (11)$$

The probability, $p_{i \rightarrow k}$, can be obtained from the dilute system (Eqn. (6)):

$$p_{i \rightarrow k} = C_0 \cdot e^{\frac{2\langle v_c \rangle \langle P_l \rangle}{D} \cdot (x_i - x_k)}. \quad (12)$$

C_0 is a calibration constant. The probability for the opposite movement is:

$$p_{k \rightarrow i} = C_0 \cdot e^{\frac{2\langle v_c \rangle \langle P_l \rangle}{D} \cdot (x_k - x_i)}. \quad (13)$$

In steady state these two rates (Eqns. (10) and (11)) are equal with opposite signs. One obtains after sorting

$$\frac{n_i}{n_0 - n_i} \cdot e^{\frac{2\langle v_c \rangle \langle P_l \rangle}{D} x_i} = \frac{n_k}{n_0 - n_k} \cdot e^{\frac{2\langle v_c \rangle \langle P_l \rangle}{D} x_k}. \quad (14)$$

This expression holds in general; it is not bound to specific k -values. Thus the left side of this equation can be replaced by a constant C .

$$\frac{n_i}{n_0 - n_i} \cdot e^{\frac{2\langle v_c \rangle \langle P_l \rangle}{D} x_i} = C. \quad (15)$$

The final result is obtained after sorting. In addi-

tion, the discrete values, x_i , on the x -axis were altered to a continuous scale, thus n_i is replaced by $n(x)$.

$$n(x) = n_0 \frac{C}{C + e^{\frac{2\langle v_c \rangle \langle P_l \rangle}{D} x}}. \quad (16)$$

The constant C can be determined by the total number of cells, N_0 .

$$N_0 = w \int_0^L n(x) dx. \quad (17)$$

For $\frac{2\langle v_c \rangle \langle P_l \rangle}{D} \cdot L \gg 1$ one obtains

$$C = e^{\frac{N_0}{n_0 w} \frac{2\langle v_c \rangle \langle P_l \rangle}{D}} - 1 \quad (18)$$

and for $\frac{N_0}{n_0 w} \cdot \frac{2\langle v_c \rangle \langle P_l \rangle}{D} \gg 1$ one obtains the final expression

$$\frac{n(x)}{n_0} = \frac{1}{1 + e^{\frac{2(x - x_G)}{l}}} \quad (19)$$

$$x_G = \frac{N_0}{n_0 w} = \frac{N_0 \cdot a_0}{w}. \quad (20)$$

This expression is identical with that one of the Fermi statistics.

For convenience let us assume that the external guiding field is applied in such a way that the cells try to accumulate at $x = 0$. The function, $n(x)$, can be described in the following way (Fig. 2): For

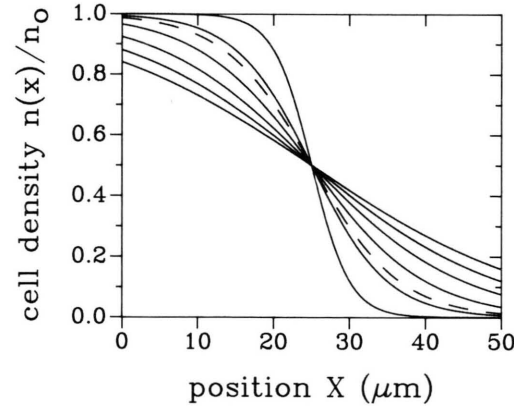


Fig. 2. Calculated normalized cell density, $\frac{n(x)}{n_0}$, as a function of position x . At a fixed boundary position ($x_G = 25 \mu\text{m}$), l was altered from 30 (flat curve) to 5 μm (steep curve) in increments of 5 μm . The dashed line is the prediction for the necrotaxis experiment ($l = 11.6 \mu\text{m}$ and $x_G = 25 \mu\text{m}$ (arbitrary)).

* The adhesion of cells to a substrate has been treaded in a similar way [12].

small x -values no free spaces are nearly absent. All sites are occupied: $n(x) \approx n_0$. On the other hand, for large x -values the cell density is very low and it decreases exponentially with increasing x as in the case of the dilute system (Eqn. (6)). The boundary between low and high density can be defined when the density is half maximum. x_G can be regarded as the mean extension of a cluster formed by migrating cells. x_G is proportional either to the total number of cells involved in the experiment or to area occupied by one cell. The slope of $n(x)$ is steepest near this boundary. If the x -position is altered from $x_G - \frac{l}{2}$ to $x_G + \frac{l}{2}$ the density changes from $\frac{n_0}{(1 + e^{-1})} = \frac{n_0}{1.37}$ to $\frac{n_0}{(1 + e)} = \frac{n_0}{3.72}$. A sharp boundary is expected for a cluster formed by granulocytes which are exposed to a strong guiding field. Such an experiment will be described in the next section.

Necrotaxis

Bessis and Nomarski [13] were the first to notice the attraction of phagocytes to dying cells. Bessis termed this type of directed locomotion necrotaxis. The necrotactic response of leukocytes is not due to bacteria-related factors, nor is it a function of the nature of the target cell. However, cells which have been dead for a long time do not act as necrotactic stimuli. The actual nature of the necrotactic stimulus is, therefore not clear [14, 15].

The signal may be chemical in nature; the resulting directed locomotion is then the chemotaxis. But to our knowledge no chemotactic molecule is known to create the necrotactic response. The signal may also be electrical in nature; the resulting directed locomotion is then galvanotaxis. For instance, when a cell is lysed, ions inside and outside the cell diffuse to reestablish a concentration equilibrium. This diffusion process is an inverse function of the size of the ions. The different diffusion coefficients of the ions involved result in the separation of small and large ions, and a diffusion potential is created. In intact cells, the plasma membrane keeps different types of ions separated from one another and thus maintains the potential difference. When the plasma membrane is disrupted, the electric field is not localized at the membrane and may be spread over a relatively large area (up to 300 μm). The strength of the electric field in the

vicinity of wounds has been measured and found to be approximately $0.2 \text{ V} \cdot \text{mm}^{-1}$. Leukocytes can orient themselves in such electric fields and thus galvanotaxis may be responsible for the necrotactic response [5].

For our experimental purposes, necrotaxis presented several advantages: (i) measurements are highly reproducible, (ii) directed and non-directed movement can be investigated before and after lysis of the target cell, and (iii) the migrating cells form a cluster since the lysed cell acts like a point source. There are also disadvantages associated with necrotaxis: the external factors are unknown as already mentioned and the magnitude of the external factors is a function of both time and space.

The model will be verified by data obtained with human granulocytes [4]. Briefly: Human granulocytes from healthy donors were investigated. Heparinized venous blood was allowed to sediment at room temperature for approximately 2 h in tubes angled at approximately 45° . The buffy coat, which consists of leukocytes-enriched plasma, was transferred to a glass slide. The suspension was covered by a coverslip and the edges sealed with paraffin. The thickness of the aqueous phase was about 20 μm . The granulocytes adhered to the surface of the glass and moved on this plane. The necrotactic gradient was created by destroying an erythrocyte with a pulsed ruby laser (694.3 nm, 3 joules in 500 μs focused to a diameter of 5 μm).

In the first part of the experiment, the diffusion coefficient and the drift velocity of the migrating granulocytes were determined in order to predict the sharpness of the boundary of the cellular cluster centered by the lysed cell. In the second part of the experiment, the formed cellular cluster was compared with the predicted distribution of the cell density.

The migration of human granulocytes can be analyzed using time-lapse micrographs (phase contrast). The center of gravity of the cells is approximated by the center of area of the cell contour line. The interval between consecutive images was 4 s. The trajectories were used to characterize the cellular responses. A sequence of pictures (Fig. 3) is shown for the necrotactic response: a) Random walk of granulocytes: the erythrocyte in the center of the picture is not yet lysed; b) 64 s after lysing the erythrocyte: granulocytes show a directed movement towards the necrotactic source;

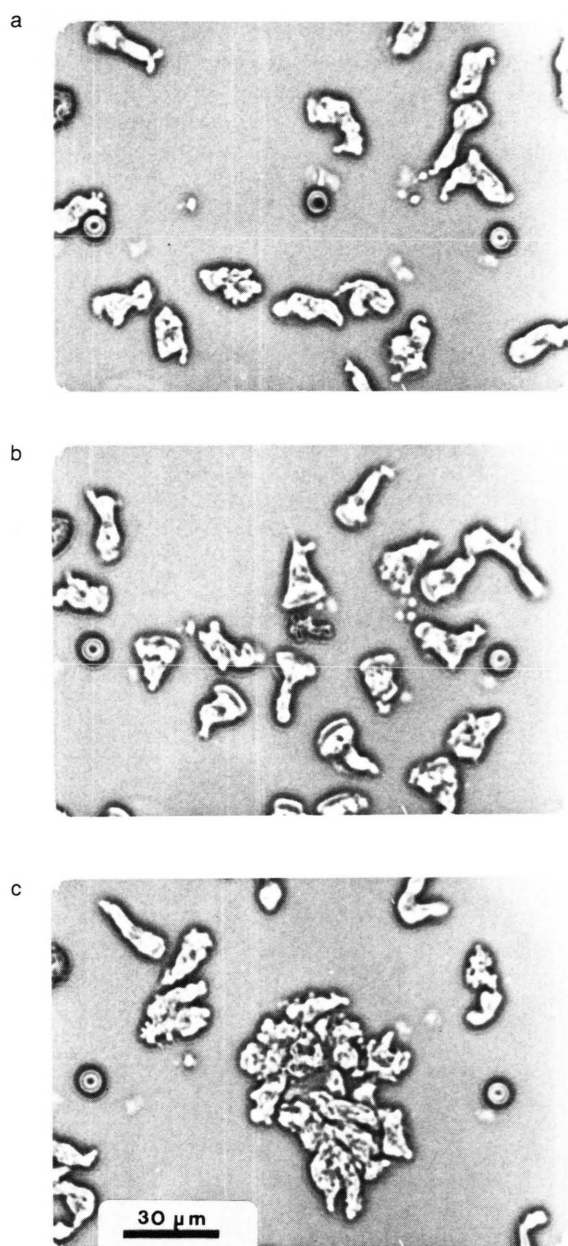


Fig. 3. Granulocytes adhering on a glass slide photographed in a light microscope (phase contrast).

a) Random locomotion, 4 s before a necrotactic source is created.

b) Directed migration, 64 s after creation a necrotactic source.

c) A cellular cluster centered by the necrotactic source. This appearance lasted between 2 and more than 6 min. The picture was taken 4 min after the creation of the necrotactic source. The cluster contained approximately 15 granulocytes. Sometime a cell joined the cluster and

c) 4 min after lysing the erythrocyte: granulocytes have formed a cellular cluster with the necrotactic source at its center. 5 to 10 min thereafter, the cellular cluster starts to desolve and finally it disappears.

Diffusion coefficient: A random walk is observed when a cell is exposed to an environment having an isotropic symmetry. This means that the migrating cell moves in a random fashion around an arbitrary chosen starting position. The average displacement is zero but the mean squared displacement is a non-zero value which can be used to quantify non-directed locomotion. A plot of the mean squared displacement as a function of time (see Fig. 6 of ref. [4]) allows to determine the diffusion coefficient D and the characteristic time τ involved in cell migration.

$$\langle x^2 \rangle = 2D(t - \tau) \text{ for } t > \tau. \quad (21)$$

We obtained: $D = 233 \mu\text{m}^2 \cdot \text{min}^{-1}$ and $\tau = 30 \text{ s}$.

Drift velocity: About 10 s after an erythrocyte is lysed, granulocytes show a strong reaction towards the lysed cell. The mean value of the drift velocity, of the polar order parameter, and of the track velocity can be determined for freely moving cells. One obtained: $\langle v_{||} \rangle = 20 \mu\text{m} \cdot \text{min}^{-1}$, $\langle P_1 \rangle = 0.84$, and $\langle v_c \rangle = 24 \mu\text{m} \cdot \text{min}^{-1}$.

The characteristic length, l , for a predicted cellular cluster is very short: $l = 9.7 \mu\text{m}$. This is approximately the size of a cell. By knowing this value we expect that the cellular cluster should have a sharp boundary: We expect that the cellular cluster formed around the lysed cell should abruptly change from close packed cells to a very dilute cell solution. A picture of a cellular cluster as shown in Fig. 3c, demonstrates that, indeed, the cell density changes within the size of a cell from a closed packed system to a dilute system.

sometime a cell left the cluster. The cells in the cluster showed still movement thus the cluster's appearance was always altering.

Conclusion

The application of the concept of steric exclusion to cell movement is very useful as it was shown. The concept is not restricted to migrating granulocytes. It can be applied to other cell types like fibroblasts, neural crest cells, *etc.* The description of the density of sterically interacting cells is closely related to the description of fermions like electrons.

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- [1] W. Alt and G. Hoffmann, *Biological Motion*. Springer Verlag, Heidelberg 1990.
- [2] P. C. Wilkinson, *Chemotaxis and Inflammation*, Churchill Livingstone, Edinburgh, London 1982.
- [3] J. P. Trinkaus, *Cells into Organs*, Pentrice-Hall Inc. Englewood Cliffs, 1984.
- [4] H. Gruler, *Blood Cells* **10**, 107 (1984).
- [5] B. Rapp, A. de Boisfleury-Chevance, and H. Gruler, *Eur. Biophys. J.* **16**, 313 (1988).
- [6] A. de Boisfleury-Chevance, B. Rapp, and H. Gruler, *Blood Cells* **15**, 315 (1989).
- [7] H. Gruler and R. Nuccitelli, in: *Ionic Currents in Development*. Ed. R. Nuccitelli, A. R. Liss, Inc., New York 1986.
- [8] H. Gruler and R. Nuccitelli, *Cell Motility and Cytoskeleton*, (1991) in press.
- [9] H. Gruler and K. Franke, *Z. Naturforsch.* **45c**, 1241 (1990).
- [10] H. Gruler, in: *Biological Motion* (W. Alt and G. Hoffmann, eds.), Springer Verlag, Heidelberg 1990.
- [11] H. Gruler and A. de Boisfleury-Chevance, *Z. Naturforsch.* **42c**, 1126 (1987).
- [12] K. Eckl and H. Gruler, *Z. Naturforsch.* **43c**, 769 (1988).
- [13] M. Bessis and G. Nomarski, *J. Biophys. Biochem. Cytol.* **8**, 777 (1960).
- [14] M. Bessis and A. de Boisfleury-Chevance, *Blood Cells* **10**, 5 (1984).
- [15] G. Gruler, *Necrotaxis and Galvanotaxis*, in: *Immunochemical Techniques. Chemotaxis and Inflammation* (G. DiSabato, ed.), Academic Press, Inc. San Diego, New York 1988.