Numerical modeling of diffusion in extracellular space of biological cell clusters and tumors.

J. Berthier, F. Rivera, P. Caillat

CEA-LETI, Département Systèmes pour l'Information et la Santé,
17, avenue des Martyrs, 38054 Grenoble, France ; jean.berthier@cea.fr

ABSTRACT

Biological cluster of cells may be seen as porous media, where the cells are the "solid grains" and the extra-cellular space (ECS) the "pores". It is often the case in dense clusters of cells that the flow rate of liquid inside the extracellular space is negligible in front of the molecular diffusion. In the particular case of tumoral cells, the extra-cellular path is the tumor interstitial matrix (IM) and the apparent (or effective) diffusion coefficient (ADC) determines the speed of delivery of drugs into the tumor. It is then of great importance to be able to estimate the value of the apparent diffusion coefficient. We present here a numerical approach, based on a Monte Carlo modeling to estimate the ADC in irregular, non repetitive morphologies of cell clusters.

Keywords: diffusion, cells, extracellular space, modeling, tumor.

INTRODUCTION

Porous media is composed of an arrangement of solid grains and interstitial fluid. Biological cluster of cells may be seen as porous media, where the cells are the "solid grains" and the extra-cellular space (ECS) the "pores" (fig 1 and 2). In fact, this is an approximation since some diffusing molecules can penetrate the cells (cellular uptake) and are thus removed from the extra-cellular space.

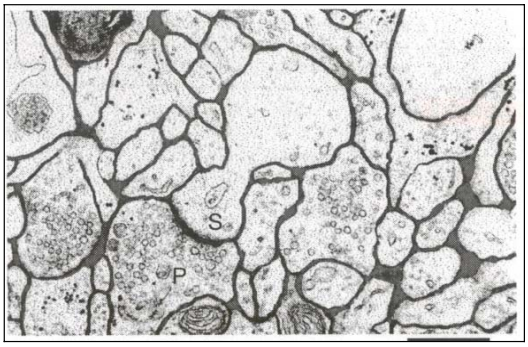

Fig. 1. Geometry of extra-cellular space from [1]. Electromicrograph of small region of rat cortex. The ECS is in dark on the picture. It can be seen at the bottom right "lakes" where the extracellular space widens.

We place ourselves in the case where the fluid flow in the ECS is negligible in front of the molecular diffusion. One example is that of tumoral cells , the extra-cellular path is called the tumor interstitial matrix (IM) [2] and the apparent (or effective) diffusion coefficient (ADC) determines the speed of delivery of drugs into the tumor [3]. It is then of great importance in cancer treatment, and to any treatment targeted at dense cluster of cells, to be able to estimate the value of the apparent diffusion coefficient [4]. It can also be noted that any change of the ADC reflects a change in the cells shape and arrangement [5].

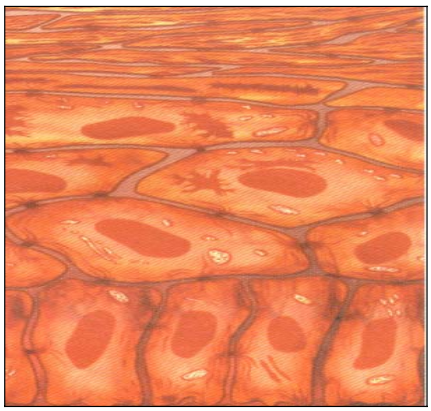

Fig. 2. Cell arrangement in the human skin from [6]. The ECS is small and the diffusion is close to the 1D case

Different types of numerical approaches have been already examined: homogenization theory [7] and Monte Carlo method [8] for regular repetitive patterns like squares or triangles. Regular patterns may seem sufficient to approximate an average ADC [9], however the recent use of microsystems to deliver drugs in-vivo requires the knowledge of diffusion in complex morphologies, specially if one wants to estimate the local uptake rates [10] or if any change in cell shape and arrangement takes place [7]. So far there have been very few investigations for irregular and disordered clusters, mostly because of the difficulty in describing the geometry. We propose here a new algorithm that includes three main features: (1) generation of a geometrical cell cluster by using the Evolver numerical program [11]; (2) a

Monte Carlo approach for calculating the random walk of the diffusing species; (3) a geometric tracking algorithm for constraining the diffusing molecules inside the ECS.

NUMERICAL MODEL

In order to investigate the diffusion process in irregular patterns of cells, we have set up a model based on a Monte Carlo approach. To the difference of the existing models, the geometry may be composed of different types of lattices of cells, not only regular but also irregular and anisotropic, mimicking real clusters of cells (fig. 3). These lattices of cells are obtained by making use of the Evolver code [11].

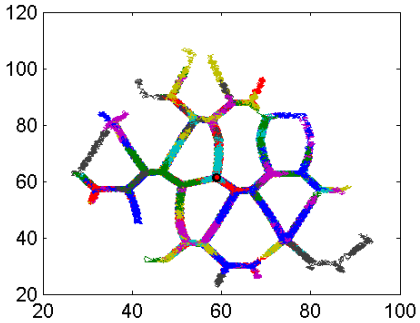

Fig. 3. A lattice of cell obtained with the Evolver code. The ECS is "painted" by the plot of the random walk of the particles.

A given set of points delimiting the cells is introduced in the Evolver numerical program in order to form an initial lattice of cells, each cell being bounded initially by linear segments. Depending on line tension and cell volume, the shape of the cells evolves until convergence to a minimum energy arrangement, mimicking real cell arrangement. It is assumed here that the cell membranes behave similarly to an interface having surface tension.
Particles are initially placed in a central micro-region, simulating the injection point at the tip of the micro-needle, the diffusion is then simulated by following the particles execute random walks inside the ECS or IM (fig.4 and 5).

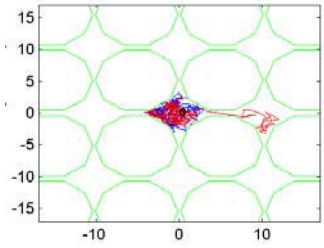

Fig. 4. Random walk of 2 particles inside the ECS of a regular lattice of rounded cells.

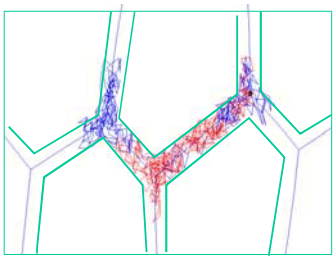

Fig.5. Random walk of 2 particles in the extra-cellular space of an irregular cluster.

The displacement (Δx, Δy) of a particle in the time step Δt is given by the relations:

$$\Delta x = \sqrt{4 D \Delta t}\cos(\alpha)$$
$$\Delta y = \sqrt{4 D \Delta t}\sin(\alpha) \qquad (1)$$
$$\alpha = random\ (0, 2\pi)$$

where D is the "free" diffusion coefficient , usually given by Einstein's law:

$$D = \frac{k_B T}{6 \pi \eta R_H} \qquad (2)$$

where k_B is the Boltzman constant (1.38 10^{-23} J/K) , T the temperature (K), η the dynamic viscosity of the carrier fluid and R_H the hydraulic radius of the particle.

Particles location inside the cluster is permanently tracked and the particles are not allowed to cross solid boundary. To this point, the difficulty is to locate each particle and to detect if it numerically crosses a cell boundary; in such a case the particle is constrained inside the ECS.

RESULTS AND DISCUSSION

We distinguish two cases: that of a cluster with small to negligible extra-cellular space and that of a cluster with free extracellular spacing (like the lattice of fig 4 and the real cells of fig.1 bottom right).
In the case of a two-dimensional array of cells with negligible intercellular spacing, the results show that there is a direct relation between the ADC and the geometrical tortuosity τ of the extra-cellular space, whatever the arrangement of the cells (fig.6 and 7). In a porous media, the geometric tortuosity is the ratio between the shortest length joining one point to another one in the fluid and the straight line. In such a case, it has been theoretically shown for a regular cell arrangement [7] that the ratio of the apparent (effective) to the free diffusion coefficient is given by $D_{eff}/D = 1/\tau^2$ where $\tau = 2^{1/2}$.

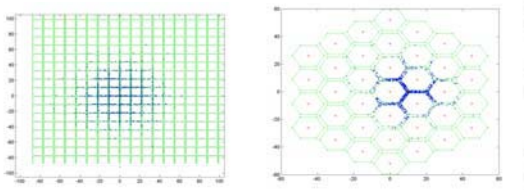

Fig. 6. (a) Diffusion in a cluster of square cells with small intercellular spacing, (b) in an hexagonal lattice.

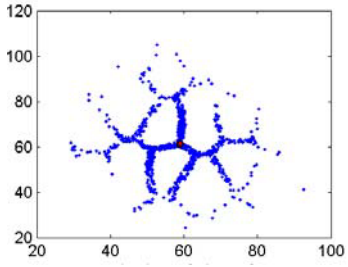

Fig. 7. Diffusion in an irregular cluster of cells with small intercellular spacing (scale in microns)

The numerical results of the present model are in agreement with this theoretical relation. In fig. 8, the normalized diffusion length

$$\beta = \frac{L}{\sqrt{4Dt}} \qquad (3)$$

(where L is obtained by averaging the distance of each particle between their location at time t and at time $t = 0$). is plotted versus time for the geometry of the cell cluster of fig. 3 and 7. At very small times, the diffusing particles execute random walk inside a small region of the ECS, so that the coefficient β is that of free diffusion: $\beta = 1$ at $t=0$. After a short time, the particles are constrained to 1D diffusion and β reaches a nearly constant value

$$\beta = \frac{L}{\sqrt{4Dt}} \approx 0.7 \approx \frac{1}{\sqrt{2}} \qquad (4)$$

By definition, the apparent diffusion coefficient satisfies

$$\frac{L}{\sqrt{4D_{eff}\,t}} \approx 1 \qquad (5)$$

From eq. (4) and (5), we find the relation

$$\frac{D_{eff}}{D} = \frac{1}{\tau^2} \approx \frac{1}{2} \qquad (6)$$

leading to the value $\tau = 2^{1/2}$.

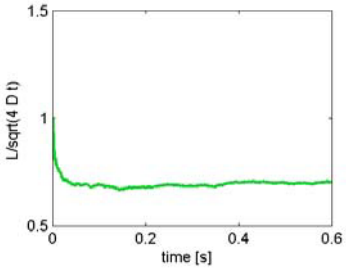

Fig. 8. Variation of the normalized diffusion distance β as a function of time, in the cluster geometry defined in figure 3 and 7.

In anisotropic clusters, tortuosity depends on the direction as shown in the figure 9 taken from [1].

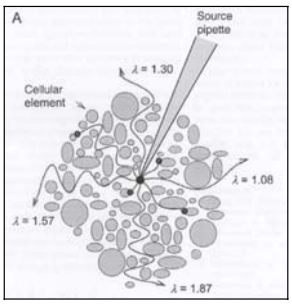

Fig. 9. Schematic view of diffusion paths in an anisotropic cell arrangement. Here λ is the tortuosity

It can be seen that even anisotropic clusters follow eq. (6) if averaging is done upon all the directions, but in such a case, it is shown that there exists a different ADC for each direction of the plane.
However, the real situation is often more complex (fig.1, bottom right) because the spacing of the cells lattice is not uniform and there are intercleft spaces. It is shown that diffusion speed may be reduced by entrapment when the dimensions of the residual spaces are large and the connecting exits sufficiently small. An idealized example is that of a cluster of round cells. If the dimension of the gaps between the cells is decreased, the apparent diffusion coefficient can be smaller than the value of eq. (6). In the case defined in fig 4., we obtain (fig. 10) $\frac{D_{eff}}{D} \approx \frac{1}{4}$.

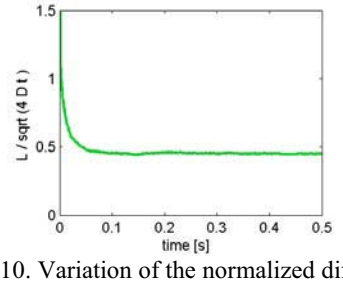

Fig. 10. Variation of the normalized diffusion distance β as a function of time, in a cluster geometry of rounded cells defined in figure 4.

A limiting case is that of a gap width smaller than the mean free path of the particle (percolation limit), in such a case, the particles are trapped inside the intercleft space.
Another example is given in fig. 11 where we have switched a cell to an intercleft space in order to compare the diffusion lengths.

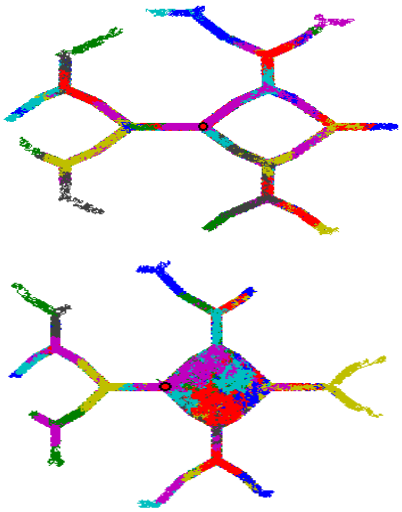

Fig 11. Random walk of particles in the ECS of a cluster of cells generated by Evolver. (a) no residual space between the cells, (b) after replacing a cell by a free space

CONCLUSION

Diffusion of biochemical species in a cluster of cells has be modelled by a three steps algorithm: (1) Evolver generation of cluster arrangement, (2) Monte Carlo random walk of the diffusing species, and (3) particle tracking to constrain the diffusing species inside the ECS.
The results of the model verify that the apparent diffusion coefficient in dense cell clusters with small extra-cellular spacing is that of the 1D case.
However the situation is much more complex in the extra-cellular space of irregular and anisotropic clusters of cells, specially if there exist intercleft spaces. Speed of diffusion can be considerably reduced by particle entrapment in the intercleft spaces.
In reality, two conditions are required for an efficient drug delivery: a diffusion speed sufficient to reach the ECS of all cells in the cluster in a reasonable time and a sufficient uptake rate to be sure that all cells are concerned by uptake.
Modeling the cellular uptake requires additional developments of the present algorithm. A very simple model for the uptake would be to introduce a probability of uptake as a function of the number of contact of the diffusing molecules on the cell membrane.

REFERENCES

[1] C. Nicholson and E. Sykova; "Extracellular space structure revealed by diffusion analysis," TINS; vol 21 n°5; 207-215; 1998.
[2] S. Ramanujan, A. Pluen, T. D. McKee, E. B. Brown, Y. Boucher, and R. K. Jain , "Diffusion and convection in collagen gels: implications for transport in the tumor interstitium," Biophys. J.; vol. 83; 1650-1660; 2002.
[3] J. Lankelma, R. F. Luque, H. Dekker, W. Schinkel, and H. M. Pinedo, "A mathematical model of drug transport in human breast cancer," Microvascular Research; vol. 59; 149-161; 2000.
[4] A. W. El-Kareh, S.L. Braunstein, T.W. Secomb. "Effect of cell arrangement and interstitial volume fraction on the diffusivity of monoclonal antibodies in tissue," Biophys. J.; vol. 64; 1638-1646; 1993.
[5] A. M. Herneth, S. Guccione, M. Bednarski, "Apparent diffusion coefficient: a quantitative parameter for in vivo tumor characterization, " European Journal of Radiology; vol 45, 208-213; 2003.
[6] M. Martin, "Conséquences d'une irradiation ionisante sur la peau humaine, " Clefs CEA, vol. 48, 53-55,2003.
[7] K. C. Chen, and C. Nicholson, "Changes in brain cell shape create residual extracellular space volume and explain tortuosity behavior during osmotic challenge, " Proc. Natl. Acad. Sci. USA; vol. 97, n° 15; 8306-8311; 1999.
[8] M. J. Saxton, "Lateral diffusion in an archipelago, the effect of mobile obstacles, " Biophys. J.; vol. 52; 989-997; 1987.
[9] J.J. Blum, G. Lawler, M. Reed, and I. Shin, "Effect of cytoskeletal geometry on intracellular diffusion, " Biophys. J.; vol. 56, 995-1005;1989.
[10] A. Szafer, J. Zhong, and J. C. Gore, "Theoretical model for water diffusion in tissues, " Magnetics Resonance in Medecine; vol. 33 n°5; 697-712; 1995.
[11] K. A. Brakke, "The surface evolver, " Experimental Mathematics, vol. 1, n°2; 141-165; 1992.