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Cell cavities increase tortuosity in brain extracellular space

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Abstract

Brain extracellular space (ECS) forms hindered pathways for molecular diffusion in chemical signaling and drug delivery. Hindrance is quantified by the tortuosity λ; the tortuosity obtained from simulations using uniformly spaced convex cells is significantly lower than that measured experimentally. To attempt to account for the difference in results, this study employed a variety of ECS models based on an array of cubic cells containing open rectangular cavities that provided the ECS with dead-space microdomains. Monte Carlo simulations demonstrated that, in such ECS models, λ can equal or exceed the typical experimental value of about 1.6. The simulations further revealed that λ is relatively independent of cavity shape and the number of cavities per cell. It mainly depends on the total ECS volume fraction α, the cavity volume fraction α_c, and whether the cavity is located at the center of a cell face or formed at the junction of multiple cells. To describe the results from the different ECS models, an expression was obtained that related λ to α, α_c, and an empirical exit factor β that correlated with the ease with which a molecule could leave a cavity and its vicinity.

Keywords: Diffusion; Extracellular space; Tortuosity; Volume fraction; Dead-space microdomain; Monte Carlo simulation

1. Introduction

Extracellular space (ECS) plays an essential role in brain functions that span long-range chemical signaling (Fuxe and Agnati, 1991; Agnati et al., 2000) to the transport of therapeutic agents (Saltzman 2001). Brain tissue is built from nerve and glial cells together with their cellular extensions. These cells, varying greatly in shape and size, are separated from each other by the narrow ECS where molecules travel predominantly by diffusion. Such diffusion encounters hindrance, quantified by the tortuosity, $\lambda = \sqrt{D/D^*}$, with D being the free diffusion coefficient and D^* being the effective diffusion coefficient in the brain (Nicholson, 2001). Tortuosity is a composite parameter that contains a significant geometrical component, although other factors, such as interstitial viscosity, may contribute. In this paper we only consider the component of tortuosity that arises purely from local geometry. For a description of the ECS geometry, an important parameter is the volume fraction, α, which is defined as the ratio of the ECS volume to the total tissue volume (Nicholson, 2001).

Early measurements of brain tortuosity often involved the perfusion of a radiolabeled compound into the ventricular spaces of an anesthetized animal and subsequent fixation of the tissue and measurement of the profiles of radioactivity (Fenstermacher and Kaye, 1988). Today, it is more common to use the real-time iontophoretic (RTI) method (Nicholson and Phillips, 1981) or the integrative optical imaging (IOI) method (Nicholson and Tao, 1993). In both the RTI and IOI techniques, substances are released from a point source and the resulting concentration distribution is measured. In the RTI method, the time course of the concentration of an ion, usually tetramethylammonium (TMA^+), is recorded with an ion-selective microelectrode at a fixed distance from the source. In the IOI method, the spatial distribution of diffusing macromolecules labeled with a

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fluorescent dye is measured at discrete times. Using the RTI method, λ and α can be measured simultaneously. Extensive use of these methods has shown that in most brain regions, $\alpha \approx 0.2$ and $\lambda \approx 1.6$ (Nicholson and Syková, 1998; Nicholson, 2001; Syková, 2004).

The theoretical calculation of tortuosity from ECS structure is a mathematical problem of a two-phase system. This type of problem was studied by Maxwell (1881) who derived a formula for the effective electric conductivity of a medium containing a dilute suspension of spheres. Maxwell's formula may be translated into a form suitable for diffusion problems with impermeable spheres (Crank, 1975), and written as

$$\frac{D^*}{D} = \frac{2}{3-\alpha} \tag{1a}$$

or

$$\lambda = \left(\frac{3-\alpha}{2}\right)^{1/2}. \tag{1b}$$

Note that the form of Maxwell's expression, as well as several others in the literature, depends on the definition of the effective diffusion coefficient; see Tao and Nicholson (2004) for further discussion.

More recently, El-Kareh et al. (1993) used homogenization theory to calculate D^* for antibodies diffusing around packed cube-shaped cells and suggested that Eq. (1b) is a good approximation for a more general class of un-elongated cells. Tao and Nicholson (2004) performed Monte Carlo simulations of diffusion in the ECS models formed by packed cubic and non-cubic cells and concluded that the geometrical tortuosity in the ECS surrounding uniformly spaced convex cells can be adequately described by Maxwell's formula (Eq. (1b)) even though the original expression was not derived for close-packed entities. From Eq. (1b), the maximum tortuosity in such an ECS is $\lambda(\alpha = 0) = \sqrt{3/2} = 1.225$, which is significantly lower than the typical experimental value of $\lambda \approx 1.6$, indicating that brain tissue cannot be treated simply as an ensemble of uniformly spaced convex cells.

Although Eq. (1b) is applicable to the ECS surrounding uniformly spaced convex cells, it does not hold for a two-phase system in general. Brown (1955) studied the dielectric constant of a two-phase material consisting of particles embedded in a medium and concluded that the dielectric constant of such a material cannot be completely determined by the volume fraction; the statistical properties of particle geometry also must be taken into account. Using variational principles, Hashin and Shtrikman (1962) determined the upper and lower bounds of the effective magnetic permeability of two-phase macroscopically homogeneous and isotropic materials, showing that Maxwell's formula is actually the upper bound. Because of the mathematical analogy, these theoretical results can by applied directly to diffusion problems, so Eq. (1b) is the lower bound for the tortuosity. Weissberg (1963) again used a variational approach to show that, in a medium containing randomly overlapping spheres, the tortuosity could be much higher than the lower bound given by Eq. (1b). It is noteworthy that these high tortuosities occur in ECS with non-uniformly spaced cells; when the volume fraction in such ECS diminishes, the non-uniform spacing will form local dead-end cavities. Such dead-end pores are known to increase tortuosity (Goodknight et al., 1960).

Recent work on brain tissue has provided experimental support for the idea that diffusing molecules are temporarily trapped in local dead-space microdomains before they exit into the main connected region and continue their travel (Hrabětová et al., 2003). This has led to the formulation of a new relation between λ and α for brain tissue with dead-space microdomains when the width of the dead-spaces is very much less than the dimension of a typical cell (Hrabětová et al., 2003; Hrabe et al., 2004, Hrabětová and Nicholson, 2004):

$$\lambda = \lambda_o \sqrt{\alpha/\alpha_o}, \tag{2}$$

where λ_o is the limiting value for a vanishingly small volume fraction, i.e. $\lambda_o = \sqrt{3/2}$ (Tao and Nicholson, 2004), α is the total extracellular volume fraction and α_o is the portion of α that would remain after elimination of dead-space microdomains.

Thus, theoretical and experimental work has indicated that, in an ECS with dead-space microdomains, the tortuosity is higher than the lower bound described by Eq. (1b). The precise geometry of the dead-spaces was not defined in the studies of Hrabětová et al. (2003) or Hrabe et al. (2004) other than to stipulate that their width should be very much less than the dimension of a typical cell. Dead-space microdomains in brain tissue might take many forms. In this study we explored the question of the relation between tortuosity and ECS geometry in more depth by taking a cell with a convex surface and forming one or more rectangular dead-end cavities, open at one end to the ECS. These pitted cells were assembled into 3-D arrays with suitable spacing to provide an ECS and then the Monte Carlo method was used to simulate diffusion in the resulting assembly. Cavity size, shape, and number per cell would be adjusted in order to answer the following questions: (1) Can the experimental value of $\lambda \approx 1.6$ be attained by introducing such cavities? (2) If so, which geometrical parameters are most important in determining λ for this model of ECS? (3) Is there a quantitative relationship between λ and these parameters for such cavities? In order to generalize our findings we considered the whole range of possible volume fractions $0 < \alpha < 1$ although the range encountered in normal and ischemic brain tissue is only $0.05 \leqslant \alpha \leqslant 0.4$ (Nicholson, 2001).