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Reduction of dimensionality in Monte Carlo simulation of diffusion in extracellular space surrounding cubic cells

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Abstract

The Real-Time Iontophoretic method has measured volume fraction and tortuosity of the interstitial component of extracellular space in many regions and under different conditions. To interpret these data computer models of the interstitial space (ISS) of the brain are constructed by representing cells as Basic Cellular Structures (BCS) surrounded by a layer of ISS and replicating this combination to make a 3D ensemble that approximates brain tissue with a specified volume fraction. Tortuosity in such models is measured by releasing molecules of zero size into the ISS and allowing them to execute random walks in the ISS of the ensemble using a Monte Carlo algorithm. The required computational resources for such simulations may be high and here we show that in many situations the 3D problem may be reduced to a quasi-1D problem with consequent reduction in resources. We take the simplest BCS in the form of cubes and use MCell software to perform the Monte Carlo simulations but the analysis described here may be extended in principle to more complex BCS and an ISS that has a defined viscosity and an extracellular matrix that interacts with diffusing molecules. In the course of this study we found that the original analytical description of the relation between volume fraction and tortuosity for an ensemble of cubes may require a small correction.

Keywords

Extracellular Space; Volume fraction; Tortuosity; Monte Carlo; Real-Time Iontophoresis

Introduction

The Real-Time Iontophoresis (RTI) method using tetramethylammonium (TMA) and other probe ions for measuring the diffusion parameters of the interstitial component of extracellular space was first described in detail by Nicholson and Phillips in 1981 [1] working in New York. The method continues to be used and the practical details have been elaborated recently [2]. Shortly after the 1981 publication, Eva Syková's group in Prague

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adopted the RTI-TMA method and software and thus began a scientific collaboration spanning almost 40 years. In 1998, Nicholson and Syková [3] summarized the diffusion work to that date and a decade later Syková and Nicholson [4] provided an in-depth survey of diffusion studies in the extracellular space.

Extensive use of the RTI-TMA methods, predominantly in the Nicholson and Syková laboratories and more recently by the Hrabová group [5], have established values for two important parameters that characterize the extracellular space, or more precisely the interstitial space (ISS): the volume fraction and the tortuosity. These parameters have been measured in different brain regions and in normal and pathophysiological conditions. Volume fraction (ϕ) is defined as the dimensionless ratio of the volume of the ISS (V_{ISS}) to the volume of the whole tissue (V_{REV}) within some Representative Elementary Volume (REV) that averages over local anatomical details, thus $\phi = V_{ISS}/V_{REV}$. Note that ϕ is used to denote volume fraction in this paper, instead of the more common α in the neuroscience literature, to avoid confusion with the letter 'a' used to denote the half-width of a cube. Tortuosity (λ) is a measure of how molecules are hindered by the geometry of the ISS and by other factors such as viscosity [6] and sometimes by fast reversible binding to fixed molecules in the extracellular matrix within the ISS [7–9]. In this paper, only geometrical contributions will be considered. Formally, tortuosity is defined by $\lambda = \sqrt{D/D^*}$ where D is the diffusion coefficient of a molecule in a free medium, typically water or very dilute gel, and D^* is the effective diffusion coefficient measured in brain tissue. If a small probe molecule such as TMA, which is largely confined to the ISS and is much smaller than the typical width of the ISS, is used with the RTI method then both ϕ and λ may be measured [1–5, 10].

The accumulation of results from use of the RTI-TMA method prompted a search for computer-based models of tissue structure that can facilitate interpretation of data and test hypotheses about the geometry of the ISS. Such models used a very simplified depiction of the brain cell, which here is called a Basic Cellular Structure (BCS). Each BCS is separated from its neighbors by an appropriate layer of ISS, the combination replicated and packed together to represent brain tissue. The resulting geometrical constructs were used in Monte Carlo simulations of diffusion to arrive at values of ϕ and λ for comparison with experimental data [8, 9, 11–15]. The initial choice for a BCS was a cube because cubes pack 3D space and may be separated by varying widths of ISS to attain any chosen value of ϕ [11]. In later work, the BCS consisted of cubes combined with cavities to simulate dead-spaces [8, 9, 13]. 'Molecules', assumed to be infinitely small, were released from a point-source in the center of the ensemble of BCS and the extent of the ensemble had to be large enough to allow the diffusing molecules to explore sufficient of the volume to establish a Gaussian distribution that could be interpreted according to the classical theory of diffusion. If the molecules did not explore enough volume, then the diffusion might be anomalous and more difficult to analyze [15].

The use of large 3D ensembles of BCS often requires a lot of computer memory, especially if the BCS had a complex geometry, and this prohibited some types of model. With repetitive structures this issue may be tackled with so-called periodic boundary conditions but this is complicated to implement [16]. In this paper we show that, by use of symmetry properties, many 3D problems may be replaced by a quasi-1D geometry that uses far less

computer memory. A second finding in this study relates to earlier work from this laboratory [11] where we found that the tortuosity in an ensemble of cubes could be described by a simple function of volume fraction that was identical to that derived by James Clerk Maxwell for diffusion in a dilute suspension of spheres. In the course of these simulations we discovered a small but systematic deviation from this ‘Maxwell’ equation.

Theory

Tortuosity value is independent of whether it is measured in 3D or 1D coordinates

In the following analysis, a 3D ensemble of cubes with associated ISS will be used to represent a block of nervous tissue. Molecules will be introduced in two different ways. The first is a ‘point-source paradigm’ meaning that molecule are released from a point-source into the ISS at the center of the ensemble of cubes mimicking the RTI [1–5, 10] or Integrated Optical Imaging (IOI) method [17]. These molecules spread out radially from the center and the diffusion equation representing the resulting concentration profile is best solved in spherical coordinates leading to an expression involving the effective diffusion coefficient (D^*) and consequently the tortuosity, λ .

A second approach is to fix the concentration of molecules at one boundary surface which causes molecules to diffuse into the cubes along one spatial dimension. This is realized experimentally by flowing a solution containing a radiotracer over the ventricular or pial surface of the brain and measuring the concentration as a function of depth in the tissue at different times. This requires a solution of the diffusion equation in a 1D coordinate system and again yields D^* and λ . This type of experiment and analysis was carried out extensively by Fenstermacher, Patlak and others [18, 19].

The 3D and 1D analyses just described involve very different source configurations and measurements in different implicit coordinate systems but, as noted by Chen and Nicholson [20], both must yield the same value of tortuosity if this parameter is going to be meaningful.

Gaussian distribution of molecules and diffusion theory for a point -source

To employ the ensemble of cubes surrounded by ISS to simulate the point-source paradigm it is only necessary to place a point-source of molecules of zero size (‘point’ molecules) in the ISS at the center of the ensemble, release M molecules instantaneously and allow the molecules to execute random walks within the connected ISS space, regarding the walls of the cubic cells as perfectly reflecting boundaries [11]. After time t , the i^{th} molecule is at position, x_i, y_i, z_i from the source. The central question is whether the distribution of positions is Gaussian, if so then it may be related to the classic diffusion equation. For a Gaussian distribution an average over all the molecular positions is expected to be zero because the molecules are released from the origin so, in order to get a useful measure, the root mean square value over the coordinates of each molecule taken separately is computed i.e. $\sqrt{\langle x^2 \rangle} = \sqrt{\frac{1}{M} \sum_{i=1}^M x_i^2}$ and similarly for $\sqrt{\langle y^2 \rangle}, \sqrt{\langle z^2 \rangle}$ over the population of M molecules.

Setting $\sigma_x = \sqrt{\langle x^2 \rangle}$ for the x -coordinates at a specific time t then the hypothesized Gaussian distribution would have the form, for a continuous range of values along the x -axis at time t :

$$G_x = \frac{1}{\sqrt{2\pi\sigma_x^2}} \exp\left(-\frac{x^2}{2\sigma_x^2}\right). \quad (1)$$

To test the validity of this distribution, a histogram could be constructed by counting all the particles in a set of intervals ('bins') of size Δx along the x -axis. Then, designating the molecule count in a given bin as M_{bin} , the prediction is:

$$M_{\text{bin}} = M \times \Delta x \times G_x \quad (2)$$

Next we establish the connection to diffusion theory by using the well-known relation between the effective diffusion coefficient for a chosen medium, D_x^* associated with the x -coordinate and time t (Eq (8) in Ref. [4] and Eq (1.10) in Ref. [21])

$$D_x^* = \langle x^2 \rangle / 2t \quad (3)$$

It follows from the definition of σ used above that $\sigma_x^2 = 2D_x^*t$ and

$$N_{\text{conc}} = \frac{M}{\sqrt{4\pi D_x^*t}} \exp\left(-\frac{x^2}{4D_x^*t}\right) \quad (4)$$

where $N_{\text{conc}} = M_{\text{bin}}/\Delta x$ is the number concentration per unit length. This is the solution to the 1D diffusion equation with a plane-source (Eq (2.6) in Ref. [22]). Note that in 1D, a plane-source is a point-source. The above analysis may be applied in each axis and could be used with anisotropic media but here an isotropic medium is assumed so

$$D^* = D_x^* = D_y^* = D_z^*.$$

To extend this argument 3D, note that, if r is the radial distance from the source at the origin, $r^2 = x^2 + y^2 + z^2$. Then $\langle r^2 \rangle = \langle x^2 \rangle + \langle y^2 \rangle + \langle z^2 \rangle$ so from Eq (3) and similar equations for the x and y coordinates:

$$D^* = \langle r^2 \rangle / 6t \quad (5)$$

(Eq 1.14 in Ref. [21]) and this may be used to estimate D^* in 3D. To establish the connection with the solution to the diffusion equation in 3D, construct a new distribution function G where $G = G_x \times G_y \times G_z$ then, by a similar argument to that outlined above, this leads to the equation:

$$N_{\text{conc}} = \frac{M}{(4\pi D^*t)^{3/2}} \exp\left(-\frac{r^2}{4D^*t}\right) \quad (6)$$

where $N_{\text{conc}} = M_{\text{bin}}/(x \cdot y \cdot z)$ is the number of molecules per unit volume and M_{bin} is now the number of molecules in a volume $x \cdot y \cdot z$. This is the solution to the diffusion equation in 3D (Eq (3.5) in Ref [22], Eq (30) in Ref [19]). Note that if the bins take the form of concentric annuli then the volume element will be $4\pi r^2 \cdot r$ and the shape of the histogram will differ from that seen in Eq (4).

Construction of a 3D ensemble of BCS and ISS

The basic representation of the tissue containing the ISS will be realized by taking a large number of BCS and packing them together in 3D with a uniform spacing that may be varied at will. Here we use the simplest BCS in the form of cubic cells with a side length of $2a$ arranged with a uniform spacing of $2w$ between them. Then each cubic cell may be envisaged as having an ‘atmosphere’ of ISS that has thickness w . Each cubic cell, together with its atmosphere of ISS, is enclosed in an imaginary bounding frame forming a cube of side $2p$ (so $p = a + w$), and an ensemble of bounding cubes forms the 3D virtual tissue (Fig. 1A). The volume fraction of the ISS is then:

$$\phi = (p^3 - a^3)/p^3. \quad (7)$$

It has been shown that results derived with ensembles of cubes appear to be identical to those derived using the more complex truncated octahedra and combinations of specific BCS, so long as the objects are convex in shape and are capable packing 3D space with an ISS of uniform width [11]. Consequently, an ensemble of cubes and their ISS suffices for the simulations discussed here. In the 3D ensemble with a point-source (Fig. 1B), D^* is obtained using Eq 5 after a steady-state condition is attained.

We already know that such a simple geometry will not generate a large enough tortuosity to match the experimental values [11, 12] and this holds true even when a component of the tortuosity is ascribed to recently measured interstitial viscosity [5, 6]. The goal in this paper, however, is to establish the validity of the reduction in the dimensionality of the model. More complex BCS, which are not convex or are packed with a non-uniform ISS [8, 9, 13] may then be substituted for the simple cubes in future models that do generate the tortuosity measured in experiments.

The quasi-1D ensemble

With modern computer workstations, Monte Carlo simulations employing tens of thousands or hundreds of thousands of cubic cells are feasible. When additional factors, such as more complex geometry, and especially the addition of molecular reactions, the time and memory required to perform the simulation in 3D may become prohibitive. To alleviate this problem resort can be made to quasi-1D geometry.

To go from the 3D ensemble to the quasi-1D geometry, we start with the 3D ensemble of cubes (Fig. 1A) and replace the point source with a plane source of molecules extending in the y - z plane and located at $x = 0$. Assuming the ensemble to be very large, all diffusion will take place in the x -axis, i.e. the diffusion problem is now a 1D problem. Strictly, in the present geometry, the penetrating cloud of molecules that emerges from between the planar

gaps oriented in the x -direction will briefly infiltrate the y - z plane but this quickly becomes a net zero motion and only occurs at the wave front of the diffusion. For this reason, however, we call this 'quasi-1D' diffusion. In the steady-state there is no net flux of molecules in the y - or z -axes so we may insert reflecting boundaries parallel to the x -axis (Fig. 1C) and in particular we may isolate a single cylinder of square cross-section containing cubes along with its atmosphere of ISS (Fig. 1D) and a plane source with M molecules at $x = 0$ (Fig. 1E).

Methods

The program MCell, version 3.2.1, 3.3 or 3.4 ([23]; www.mcell.org) was used to perform the Monte Carlo simulations using either the Linux or Windows versions running on a variety of quad-core Intel processors. Calculation of initial geometry parameters for the MCell script and analysis of the MCell output, as well as the figures, was accomplished with custom programs written in MATLAB.

In a prior study [11] we determined D^* and λ by counting the number of molecules in a set of concentric boxes arranged symmetrically around the point-source. Here we used a more sensitive measure of these diffusion parameters by computing the mean square distance from the source of every diffusing molecule at a sequence of times (Eqs 3 and 5).

Most studies with 3D ensembles of cubes employed 64x64x64 cubic cells, i.e. a total of 262,144 cubes, with half width $a = 0.25 \mu\text{m}$. When this value of a was used together with a volume fraction of $\phi = 0.2$, a value $w = 19.3 \text{ nm}$ was obtained, so the width of the ISS was 38.6 nm, which agreed with the estimate of 40 nm for an ISS comprised of sheets or parallel planes obtained with quantum dot nanoparticles using Integrated Optical Imaging (IOI; [5, 24]). Much of the detailed analysis was carried out with these parameters, which most nearly approximate our present thinking about brain tissue values. To further compare results between the 3D and quasi-1D models we used a set of specified ϕ -values (0.1, 0.2, 0.4, 0.6, 0.8 and 0.95) then Eq (1) is rearranged to obtain a set of p , keeping $a = 0.25 \mu\text{m}$:

$$p = a(1 - \phi)^{1/3}. \quad (8)$$

The value of $w = p - a$, the width of the ISS, then varies accordingly.

A total of 100,000 molecules were released instantaneously from a point-source at the center of the ensemble (Fig. 1B), moving in the ISS with free diffusion coefficient $D = 1 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. The value of D was chosen to represent that for a typical small molecule; as an example, for TMA^+ , $D = 1.11 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ at 37°C [4]. The results are independent of the actual value of D but a smaller diffusion coefficient will mean that it will take longer for the molecules to explore the geometry, which will be compensated by the ability to use a larger time-step in the simulation. Time steps were chosen so that mean radial displacement ($\bar{l}_r = 4\sqrt{D\Delta t/\pi}$, Eq (4.6) in [23]) was much smaller than $2w$. The time step used in most simulations was $t = 20 \text{ ns}$ except for the runs for $\phi = 0.1$ where a time step of 5 ns was employed because of the narrower ISS. Simulations for $\phi = 0.2$ were repeated with a time step of 2 ns without any appreciable difference in the results. The value of D^* was estimated from Eq (5) and then the tortuosity calculated as $\lambda = \sqrt{D/D^*}$. In reality, λ is time-dependent

during much of the early time after molecules are released so the simulation was continued until $\lambda(t)$ appeared constant (see Results for discussion of ‘transient tortuosity’) and then the value of λ was averaged over an appropriate set of output iterations. The typical duration of the simulation was 0.008 s and a check was made that no molecules had left the ensemble. Because of the stochastic nature of the simulation, for each chosen geometry, four independent Monte Carlo simulations were run with different initial random number seeds (seeds 1–4 in MCell), the results averaged, and the standard deviation (SD) calculated.

For the quasi-1D model (Fig 1B), a single row of cubes was enclosed in a square bounding tube of cross-section $2p \times 2p$. As described above, the walls of the bounding tube were reflecting. The values of D^* and were calculated from Eq (3) and the overall protocol of the simulation was similar to that described for the 3D study.

Results

Diffusion in a 3D ensemble of cubes

The molecules are released from a point source in the center of a 3D void formed by the eight cubes (Fig. 1B) and their associated ISS in the center of the larger ensemble of $64 \times 64 \times 64$ cubic cells. A reduced ensemble of $8 \times 8 \times 8$ cubes is shown in Fig. 1A. The molecules form a cloud that diffuses out through the ISS of the ensemble of cubic cells and an effective diffusion coefficient may be computed using Eq (5). This parameter, however, will be potentially time dependent whereas the definition of D^* calls for it to be a constant in order that the classic diffusion equation is a valid representation of the physical process. Equally, the definition of λ demands that it is constant too. Therefore, it is useful to define a parameter called the ‘transient effective diffusion coefficient’, D_t^* , operationally derived from Eq (5) and the corresponding ‘transient tortuosity’ λ_t .

$$\lambda_t(t) = \sqrt{\frac{D}{D_t^*}} = \sqrt{\frac{6Dt}{\langle r^2 \rangle}}. \quad (9)$$

It will be shown from the experimental data that, for sufficiently large t , $D_t^* \rightarrow D^*$, and $\lambda_t \rightarrow \lambda$.

The parameter, $\lambda_t(t)$, defined by Eq (9), is computed from the position of every molecule in the diffusing cloud at sequence of time steps. A typical plot with a logarithmic time axis is seen in Fig. 2A; the inset plot shows the same data on linear time axis that compresses all the early time behavior so much that no detail is visible. Fig. 2A was derived from the first of the four simulations comprising Run Set 2 in Table 1 and used Seed 1 in the MCell software. The plot may be divided into three phases based on the time epoch.

Phase I—Immediately after release, up to the time the molecules encounter one of the cube surfaces, they undergo free diffusion with diffusion coefficient $D = 1.0 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. This free diffusion phase is very brief and ends at time t_1 , about $0.1 \text{ } \mu\text{s}$ (Fig. 2A). During this phase $D_t^* = D$ so that $\lambda_t = 1$; both these parameters are constant, and the diffusion equation is valid. Thereafter, the molecules enter Phase II where they are exploring the local geometry.

Phase II—The increasing number of collisions with the walls of the cubes causes a steady increase in tortuosity. During this phase D_t^* is an increasing function of time (Fig. 2A) so it no longer satisfies the classic diffusion equation; whether it may be defined as anomalous diffusion [25] will not be discussed here. In principle, this monotonic increase in tortuosity could persist indefinitely, which would render moot the analysis of tortuosity, but it does not. Instead, a plateau is reached, designated as Phase III.

Phase III—This phase begins at about 2 ms (this is a conservative estimate based on Fig 2A together with an examination of data from the three other MCell simulations used in Run Set 2 shown in Table 1) indicating that the molecules have explored ‘sufficient’ geometry to provide a stable value for λ . The distribution of molecules at t_2 in the x - y plane in a thin slice centered on $z = 0$ and spanning $z = \pm 0.45 \lambda\text{m}$ is shown in Fig. 2B. This plateau phase continues for the duration of the MCell run, which is 8 ms (0.008 s) here and in most other runs. During Phase III, the distribution of molecules is Gaussian in all axes, as shown in Figs. 2C–E. This means that during Phase III and beyond, Fick’s Second Law, i.e. the diffusion equation, may be applied as though we are dealing with a free medium with a new, constant effective diffusion coefficient. For the data shown in Fig. 2A,

$D^* = D_t^* = D/\lambda^2 = 7.05 \times 10^{-6} \text{ cm}^2\text{s}^{-1}$ and $\lambda = \lambda_t = 1.191$ (cf. mean $\lambda = 1.192$ for Run Set 2, Table 1).

Diffusion in a quasi-1D ensemble of cubes

In the quasi-1D model there is a single row of 64 cubes bounded by reflecting walls and centered on the x -axis on either side of $x = 0$; a row of eight cubes is shown in Fig. 1D. The molecules are released from a thin sheet $2w/100 = 0.386 \mu\text{m}$ in width in the y - z plane centered at $x = 0$ (Fig. 1E). The molecules diffuse in both the positive and negative directions in the x -axis and the distributions are added. Again, it is desirable to define a parameter called the ‘transient effective diffusion coefficient, D_t^* , operationally defined by Eq (3), and the corresponding ‘transient tortuosity’ λ_t .

$$\lambda_t(t) = \sqrt{\frac{D}{D_t^*}} = \sqrt{\frac{2Dt}{\langle x^2 \rangle}} \quad (10)$$

Plotting λ_t versus t on a logarithmic scale in Fig 3A, using the same values for a , w and D as were employed in the 3D simulations, and corresponding to the first simulation (MCell seed 1) from Run Set 7 in Table 1. Again, there are three phases in the behavior of λ_t .

Phase I—This phase lasts until about 20 ns (t_1 in Fig 3A) and represents the time in which molecules move from their initial location in the thin plane-source to the surfaces of the cubes bounding the ISS at the release site. By 20 ns, the gap between the cells bounding the plane at $x = 0$ has filled with molecules and λ_t begins to climb in value as Phase II commences.

Phase II—This spans the interval from $t_1 = 20 \text{ ns}$ to $t_2 = 2 \text{ ms}$ (see Fig 3A). In contrast to monotonic rise of λ_t seen during Phase II in the 3D case, Phase II in the quasi-1D simulation

reaches a peak value of $\lambda_t = 1.16$ at $t_p = 1.5 \mu\text{s}$ (Fig 3A) then falls to a plateau value at around 2 ms. The inset plot in Fig. 3A shows the distribution of molecules at t_p and shows they are just beginning to leave the confined space between the cubes on either side of $x = 0$. This also provides an explanation for the rising tortuosity up to that time; the molecules are confined to the region $-w - w$ so $\langle x^2 \rangle$ is essentially constant while time steadily advances leading to the rise in λ_t calculated according to Eq (10). As molecules leave the gap between the center cubes, λ_t falls, eventually reaching a steady-state value, which implies insignificant net molecular movement in the y - z planes and all net molecular movement is in the x -axis.

The explanation of the transient increase in tortuosity seen during Phase II was confirmed by releasing all the molecules from a point-source located at the origin that lay at the center of the y - z plane between the two central cubes (Fig. 3B). This simulation had the same parameters as the four used for Run Set 8 in Table 1 but to obtain the time resolution needed to construct the plot, the time step was reduced to 2 ns and MCell Seed 1 was used. Like the planar source, the point-source leads to an initial Phase I of duration $t_1 = 20$ ns where the molecules undergo free diffusion with $\lambda_t = 1$ then tortuosity begins to rise (Fig. 3B). The molecules are now confined to the thin region between the cubes for longer than when the source is a sheet of molecules and the increase in λ_t is correspondingly greater (Fig. 3B), peaking at $t_p = 5$ s where $\lambda_t = 7.6$, which is both larger and later than what was observed in Fig 3A. Again, a snapshot of molecular distribution (inset, Fig. 3B) shows that peak λ_t occurs as the molecules start to leave the confined space between the cubic cells bounding the source. Thereafter λ_t falls to the same plateau value as seen in Fig. 3A at the same time ($t_2 = 2$ ms) showing that the long-time behavior of diffusing molecules after instantaneous release ‘forgets’ the shape of the initial distribution.

Phase III—This phase appears to begin at 2 ms for both plane- and point-source (Figs. 3A, B), like the Phase III associated with the 3D simulation. Thereafter there is a constant effective diffusion coefficient and $D^* = D_t^*$, consequently $\lambda = \lambda_t = 1.195$ for the simulation shown in Fig. 3A and $\lambda = \lambda_t = 1.198$ for the simulation shown in Fig 3B. The mean tortuosity for four simulations with the planar source (Run Set 7, Table 1) was $\lambda = 1.195$. For the point source (Run Set 8, Table 1) the mean was $\lambda = 1.194$.

The distribution of molecules now closely conforms to a Gaussian for both sources (Eq. 4; Figs. 3C, 3D) verifying the validity of the diffusion equation for $t > t_2$. The spatial picture of the molecular distribution is shown below Panels C, D in Fig. 3 for molecules at $t_2 = 2$ ms in the x - y plane in the volume defined by $z = \pm 0.45 \mu\text{m}$. This slice includes the molecules in the central ISS plane together with molecules in the gaps between adjacent cubic cells.

Comparison of 3D and quasi-1D simulations for $\phi = 0.2$

Table 1 shows details of several run sets with 3D and quasi-1D geometry. Overall the results are the same for both geometries with λ in the range 1.192–1.195 with a median of 1.935. Each λ -value is the average of four simulations with MCell seeds 1–4. The SD based on these sets of four simulations is smaller for the 3D runs shown here (Run Sets 1–3) compared to the quasi-1D runs (Run Sets 5–8). This change reflects the slightly greater

variation in the results with the much smaller number of cubes in the quasi-1D case. Nevertheless, the quasi-1D geometry is entirely useable and requires much less computer memory.

Run Set 4 employs a 3D geometry, but the parameters are those used in the original study by Tao and Nicholson [11] with only 1000 molecules thus accounting for the greater SD. It is also noteworthy that the typical time for a simulation in Run Set 4 was 12 minutes on a present-day personal computer compared to a time of “a few hours to a couple of days” in the 2004 study [11] reflecting the huge advance in computing power over these 14 years. Looking at Table 1 it is also evident that differences in cube size have no effect on the results confirming that tortuosity in this geometry is only a function of volume fraction. Finally we showed (compare Run Sets 7 and 8) that the use of either a sheet-source or a point-source leads to similar λ -values in the quasi-1D simulations, although the early time transient behaviors are different (see Figs. 3A, 3B).

Comparison of 3D and 1D simulations over a range of ϕ

A plot of the tortuosities as a function of volume fraction is shown in Fig. 4A. It is known that for packed cubes $\lambda = \sqrt{3/2}$ when $\phi = 0$ [26, 27] and obviously $\lambda = 1$ when $\phi = 1$, therefore these two ‘end points’ were added to the plot. It is apparent that the λ versus ϕ data points for the 3D and quasi-1D models are almost identical thereby further validating the use of the quasi-1D model.

Fig. 4A also plots the ‘Maxwell’ function λ_M ,

$$\lambda_M = \sqrt{(3 - \phi)/2} \quad (11)$$

that was previously found to accurately describe 3D cubic cell results [11]. Unexpectedly, we found a systematic deviation from the Maxwell function in our present results. The deviation is quite small but may be amplified by subtracting λ_M from each of the MCell results, i.e. plotting

$$g = \lambda - \lambda_M \quad (12)$$

for each of the given set of ϕ . These plots are shown for the 3D results in Fig. 4B and for the quasi-1D results in Fig. 4C, which also shows the SD values derived from four MCell simulations averaged for each data point. Based on polynomial fitting to data points for the 3D results it was found that $g_{3D} = 0.07\phi(1 - \phi)$ well described the deviation from the Maxwell equation (Fig. 4B) and was adequate for the quasi-1D results (Fig. 4C). There is more variability in the quasi-1D result compared to the 3D indicating another tradeoff in using the much smaller number of cubes; the variability could likely be reduced by averaging more MCell simulations with more starting seeds. Nevertheless, we suggest that the equation

$$\lambda = \lambda_M + g_{3D} = \lambda_M + 0.07\phi(1 - \phi) \quad (13)$$

may be a useful correction when high accuracy is required for an ensemble of cubes.

Discussion

We have shown that, in a symmetrical 3D ensemble involving BCS, the problem of diffusion in the ISS may be replaced by a quasi-1D problem with a large saving in computer memory and some saving in time. There is some cost insofar as more repetitions of the simulations may be required in the 1D case to obtain a similar SD for the tortuosity as in the 3D case. These advantages are not so apparent in the very simple BCS used here – a cube – but more complex structures, such as cubes with cavities at all corners [9, 28] demand considerably more computer memory. The real advantage, however, may become apparent when reaction kinetics is added, for example reactions with extracellular matrix [9, 28] that make heavy demands on computer resources.

These studies also confirm that the tortuosity generated by ensembles of cubes are scale independent and independent of the free diffusion coefficient used (Table 1). They also demonstrate that a certain time after molecules have left a source, the source geometry ceases to affect the distribution (compare Figs 3A, 3B).

The time evolution of effective diffusion coefficient, D^* , and hence, λ goes through three phases in both 3D and 1D models. During the first phase, measured nanoseconds, there is free diffusion before the released molecules reach a cell wall. Thereafter, there is a second phase where molecules begin exploring the structure and the calculated D^* is time-dependent and not a true diffusion coefficient. This is especially pronounced in the quasi-1D model. But after about 2 ms, with the parameters used here, the distribution of molecular positions conforms to a Gaussian and the diffusion equation is obeyed from then on. To put this in perspective, the response time of a conventional ion-selective microelectrode capable of sensing TMA, as used in the RTI method, is of the order of tens of milliseconds [29].

Finally, we were surprised to see the deviation from the ‘Maxwell’ equation as shown in Fig. 4. It would be interesting to know if it occurred with the ensembles of truncated octahedra and mixed shapes used by Tao and Nicholson [11]. It is not a function of the cube size because Table 1 shows that the same tortuosity is obtained for $\phi = 0.2$ with a variety of cube sizes. We have made MCell simulations with $a = 0.9 \mu\text{m}$ for other values of ϕ and obtained similar values to those shown in Fig. 4A (data not shown). Looking carefully at Fig. 9 in Ref. [11] it is possible to see a hint that most data points are above the λ_M plot but the variability associated with these early runs, which used only 1000 molecules, obscured the deviations. From another perspective, the fact that the same deviation occurs with both the 3D and quasi-1D simulations supports the equivalence of the two geometries. The reason for the deviation is not obvious at this time and will require further analysis; it is noteworthy that it appears to peak at $\phi = 0.5$. One clue to origin of the deviation might be found in the study of Bell and Crank [30] who provided an analytical solution together with numerical evaluation for diffusion in a 2D ensemble of cubes. They showed that the concentration profiles in the gaps between cubes was not uniform but was influenced by presence of sharp corners and the arrangement of the cubes. A similar finding is evident in the 2D study of Chen and Nicholson [20] using a wider variety of shapes. How these results translate to 3D and whether they are the cause of the deviation remains to be determined.

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Figure 1.

The simulation models as 3D and quasi-1D ensembles of spaced cubes. **A.** Ensemble of $8 \times 8 \times 8$ cubes with sides $2a = 0.5 \mu\text{m}$ separated by ISS of width $2w = 38.6 \text{ nm}$. This geometry corresponds to $\phi = 0.2$. The actual simulations most often used $64 \times 64 \times 64$ cubes. **B.** Molecules (red in online version) are released instantaneously from a point-source in the ISS at the center of the ensemble of cubes. Here the center eight cubes are shown as transparent with the distribution of 100,000 molecules $1 \mu\text{s}$ after release. The molecules are actually confined to the narrow planes between the cubes. **C.** Transition from 3D to quasi-1D. Taking a plane through the ISS at the center of the ensemble shown in Panel A and expanding the point into a plane source forces a quasi-1D diffusion (see text) allowing a single column of cubes and their surrounding ISS (dashed lines) to be isolated and used to model the diffusion problem. **D.** Isolated column of cubes and bounding atmosphere of ISS shown as wire frame (green in online version). **E.** shows center two cubes with distribution of 100,000 molecules

from the plane-source 1 μ s after release as molecules are starting to leave the initial confinement between the cubes where the plane-source was located.

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Figure 2.

Evolution of tortuosity to steady-state value in 3D ensemble of cubes from point-source release for model with $\phi = 0.2$. **A.** Transient tortuosity (λ_t , Eq (9)) as a function of logarithmic time. From the initial release of molecules to time t_1 there is free diffusion, from t_1 to t_2 the value of λ_t changes continuously. After $t_2 = 2$ ms, λ_t is constant. Inset plot shows the same curve on a linear time scale where the early-time behavior cannot be resolved. **B.** The distribution of molecules in a slice $\pm 0.45 \mu\text{m}$ above and below $z = 0$ in the x - y plane. This includes molecules in plane at $z = 0$, which is $2w = 38.6 \mu\text{m}$ wide and the vertically oriented space between the cubes. **C, D, E.** Histograms of molecule x , y , z coordinates at t_2 and Gaussian curves according to Eq (4). Bin width is $0.6 \mu\text{m}$.

Figure 3.

Evolution of tortuosity to steady-state value in 1D ensemble of cubes from plane- and point-source release for model with $\phi = 0.2$. **A.** Planar source (see text). Transient tortuosity (λ_t , Eq (10)) as a function of logarithmic time. From the initial release of molecules to time t_1 there is free diffusion, from t_1 to t_2 the value of λ_t changes continuously. In contrast to the 3D case, molecules remain between walls of two cubes for some time and λ_t rises until time $t_p = 1.5 \mu\text{s}$ when molecules begin to escape (see inset). As the molecules enter the larger geometry, λ_t falls then plateaus and after $t_2 = 2 \text{ ms}$, λ_t is constant. **B.** Point source (see text). Transient tortuosity (λ_t , Eq (10)) as a function of logarithmic time. The trajectory of the curve is similar to Plot A but the peak in λ_t is larger because it takes longer for molecules escape from their initial confinement between walls of the cubes. The escape begins at $t_p = 5 \mu\text{s}$ (see inset). As the molecules enter the larger geometry, λ_t falls then plateaus and after $t_2 = 2 \text{ ms}$, λ_t is constant. **C.** Histogram of molecular positions at time t_2 for the plane source used in Plot A. Dashed line is Gaussian curve (Eq 4). Bin size $0.6 \mu\text{m}$. Inset below shows molecular positions at time t_2 in a slice $\pm 0.2 \mu\text{m}$ wide. **D.** Histogram molecular positions at

time t_2 for the plane source used in Plot B. Dashed line is Gaussian curve (Eq 4). Bin size $0.6\text{ }\mu\text{m}$. Inset below shows molecular positions at time t_2 in a slice $\pm 0.2\text{ }\mu\text{m}$ wide.

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Figure 4.

Tortuosity as a function of volume fraction. **A.** Results for 3D model (open circles) and quasi-1D model (crosses). Each data point is mean of four MCell runs with starting MCell seeds 1–4. Known end points (squares; see text) are inserted. The theoretical Maxwell equation (λ_M ; Eq (11)) is shown as a line. It is seen that the two models produce almost identical tortuosity values for each value of ϕ but there is a systematic deviation from the λ_M curve. **B.** The deviation is made more evident by subtracting λ_M from each λ -value. The error bars are the standard deviations for the four runs comprising each data point. The curve is $g_{3D} = 0.07\phi(1-\phi)$ which is the best quadratic fit to the data points. It is seen that the deviation curve is symmetrical with the greatest deviation occurring at $\phi = 0.5$. **C.** A similar plot to that shown in Plot B for the quasi-1D data. The same function g_{3D} is superimposed to show that the fit remains quite good; however the standard deviations are mostly larger in

the quasi-1D case compared to the 3D model. All models based on cubes with sides $2a = 0.5$ μm with ECS half width w varied to obtain required value of ϕ .

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Table 1.Simulations with volume fraction, $\phi = 0.2$, in 3D and quasi-1D geometries

Run Set	Dim	Source	Ensemble	$2a$	$2w$	Molecules	$D \times 10^5$	t	Dur	$\lambda(N=4)$	Clock
				μm	nm		$cm^2 s^{-1}$	ns	s	$mean \pm SD$	hr
1	3	Point	64x64x64	0.5	38.6	100,000	1.0	20	0.008	1.194 ± 0.001	7
2	3	Point	64x64x64	0.5	38.6	100,000	1.0	2	0.008	1.192 ± 0.001	56
3	3	Point	64x64x64	1.9	147.0	25,000	1.0	20	0.004	1.192 ± 0.003	9
4	3	Point	20x20x20	2.785	215.0	1000	0.1	1000	1.000	1.194 ± 0.014	0.2
5	1	Plane	64x1x1	0.5	38.6	100,000	1.0	20	0.008	1.195 ± 0.003	5
6	1	Plane	128x1x1	0.5	38.6	100,000	1.0	20	0.016	1.193 ± 0.002	10
7	1	Plane	64x1x1	0.5	38.6	100,000	1.0	2	0.008	1.193 ± 0.002	30
8	1	Point	64x1x1	0.5	38.6	100,000	1.0	20	0.008	1.194 ± 0.006	4

Each Run Set comprises four MCell simulations. Dim is dimension of ensemble ('1' refers to quasi-1D). Source is geometry of release site. Ensemble is configuration of cubes, each surrounded by ISS of width w . $2a$ is width of cube. $2w$ is width of ISS. Molecules is number of molecules released from source. D is free diffusion coefficient. t is time-step used in MCell simulation. Dur is duration of the simulation in model time. λ , tortuosity, is average obtained from four runs with MCell seeds 1–4 and measured after a steady-state value was attained (for comparison, $\lambda_M = 1.183$ for $\phi = 0.2$). Clock is the typical laboratory time it took to complete one run. Where applicable, units are listed in the second row of the table.