Simulation of Transport of Lipophilic Compounds in Complex Cell Geometry

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Abstract: The mathematical modeling of the diffusion and reaction of toxic compounds in mammalian cells is tough task due to their very complex geometry. The heterogeneity of the cell, particularly the cytoplasm, and the variation of the cellular architecture, greatly affects the behavior of these toxic compounds. Homogenization techniques have been implemented for the numerical treatment of the model. This technique considerably reduces the complexity of the model. To see the behavior of the cell after the inclusion of the toxic compounds, we have implemented a mathematical model in Comsol Multiphysics. The results of the model have been validated against the results got from the experiments performed in vitro. The work has been done in 2-dimensional space and can be extended to 3-dimensional space and also with more complex reaction systems.
Keywords: Complex cellular architecture, Reaction and Diffusion system, Homogenization, Metabolism in biological cells.

1. Introduction

A cell is a fundamental unit of living organisms. Schematically, a human cell consists of outer cellular membrane, cytoplasm that contains lot of cell organelles i.e. mitochondria, Golgi apparatus, endoplasmic reticulum etc, then a nuclear membrane and finally a nucleus containing the most important hereditary material DNA. The mathematical description of the biotransformation in the cell shows a very complex reaction-diffusion system due to the presence of many thin membrane structures. The membranes of cell organelles create a very dense and complex system throughout the cytoplasm. If these are considered to be separate sub-domains, then the model will become very complex and computationally expensive. Moreover, due to the natural variations in the cell structures, every cell needs to have its own mathematical model. The effect of drug diffusion in different cell geometries can be seen in [2]. In the presented model, we will be able to analyze the behavior of chemicals in the cell. Later on, this approach will be very useful in the modeling of complex geometrical cell and reaction systems. In order to validate the specific model and mathematical approach, we have collected sets of data from in vitro experiments and cells in culture describing the partitioning, intracellular metabolism, and reactivity of polycyclic aromatic hydrocarbons (PAHs) [3-7].

For the numerical treatment of the model without changing the essential features of metabolism, the approach for homogenizing the cytoplasm has already been used in [10,11] using periodic homogenization, but in this paper we will use iterative homogenization including periodic and stochastic homogenization which has been done in [1]. For our modeling strategy, we will use the assumptions in [8], which are summarized here:

• On a small scale in space, the volume between the outer cellular membrane and the nuclear membrane consists of layered structures/membrane.

• In a large scale, this volume contains an unordered set of the small-scale substructures which are uniformly distributed over the volume.

• The physical and chemical properties of the cytoplasm and of the membranes are uniform.

• We adopt the continuum hypothesis, i.e., we assume that the set of molecules in the cell can be modeled by considering a continuous representation (a concentration).

• Absorption and desorption is in rapid equilibrium at the membrane/cytoplasm boundary and therefore the relative concentration at the border can be conveniently described by the partition coefficient.

In the 2nd section, we will introduce the mathematical model of our system. In the 3rd section, we will discuss on the different cellular architectures. In the last section, we discuss the modeling in Comsol Multiphysics and the numerical results.

2. Mathematical Model

2.1 Model Description

This model describes the uptake of different chemicals into the mammalian V79 cells. Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants formed from incomplete combustion. PAH can be metabolized to reactive intermediates that react with protein and DNA and thereby cause toxicity and cancer. Different PAH form a large variety of reactive intermediates of which some are more or less carcinogenic. In order to understand the major principles that govern cellular fate of reactive intermediates formed from PAH, and why some are carcinogens and others not, we have developed a computational tool to model the diffusion and reactions taking place. In our system, the ultimate reactive

metabolite is modeled in terms of uptake into cells, diffusion within cells and intracellular membranes, hydrolysis, conjugation and DNA binding. Uptake and diffusion as well as partitioning into membranes are physical processes whereas hydrolysis of the reactive intermediate or reactions with DNA are chemical processes. In addition, conjugation of reactive intermediates to glutathione catalyzed by enzymes called glutathione transferases is an important defense mechanism in living cells. The key step for developing cancer is covalent binding of reactive intermediates to DNA. The extent of DNA binding is determined by uptake, diffusion, partitioning, hydrolysis, conjugation and DNA reactivity. These factors are in turn determined by the physicochemical properties of the reactive intermediates and the ability of the glutathione transferases to elicit efficient conjugation. In addition cellular architecture might play an important role in dictating the amount of reactive intermediate that is able to reach DNA which is located centrally in the nucleus. These studies are performed to determine the physicochemical characteristics of efficient carcinogens and the importance of cellular factors. In addition our model can serve as a tool to model the fate of lipophilic compounds in cells contributing to cellular modeling [2].

2.2 Quantitative Model

For the analysis of reactions and diffusion in the cells, we have already described the Mathematical model in [1,2]. In order to distinguish between the concentrations within the different sub-domains, we add an index. For example, we denote C in nucleus by C_5 .Similarly the diffusion coefficient will also denoted by $D_{index\ of\ domain}$. The following diagram shows the complete reaction and diffusion system along with the five sub-domains of the cell [2].

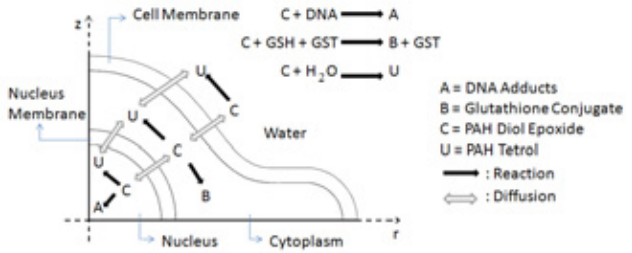

Figure 1. Diagram of quarter Part of an axi-symmetric cell (not to scale) displaying reactions and diffusion in and around a cell.

The above reaction-diffusion system gives rise to the following partial diffusion equations.

• **Sub-domain 1 (extracellular medium)** Since we have the following chemical reaction in this domain

$$C \xrightarrow{k_U} U$$

and C,U diffuse through the domain, the reaction and diffusion give rise to the following PDEs

$$\frac{\partial C_1}{\partial t} = \nabla.(D_1 \nabla C_1) - k_U C_1 ,$$
$$\frac{\partial U_1}{\partial t} = \nabla.(D_1 \nabla U_1) + k_U C_1 .$$

• **Sub-domain 2 (cell membrane)** Since in this domain C,U take part in diffusion process, and there is no chemical reaction,

$$\frac{\partial C_2}{\partial t} = \nabla.(D_2 \nabla C_2) ,$$
$$\frac{\partial U_2}{\partial t} = \nabla.(D_2 \nabla U_2) .$$

• **Sub-domain 3 (cytoplasm)** Here we have the following reactions

$$C \xrightarrow{k_U} U ,$$
$$C \xrightarrow{k_B} B .$$

Only C,U are subject to diffusion. Hence, we have the following PDEs

$$\frac{\partial C_3}{\partial t} = \nabla.\left(D_{3,\text{eff}} \nabla C_3\right) - (k_U + k_B) C_3 ,$$
$$\frac{\partial U_3}{\partial t} = \nabla.\left(D_{3,\text{eff}} \nabla C_3\right) + k_U C_3 ,$$
$$\frac{\partial B_3}{\partial t} = k_B C_3 .$$

Since the geometry of the cytoplasm is very complex, so to resolve the small geometric structures, we need a very small grid, otherwise the computational cost will be very high. To avoid this, the effective equations were used and homogenization techniques were adopted in [1]. The process of the derivation of the homogenized model of cytoplasm has been very extensively described in [1], which uses the following steps:

• Find an effective diffusion coefficient, $D_{3,\text{eff}}$ for the homogenized cytoplasm.

• Modify the reaction terms and time constant (in such a way that only partial concentrations are taken into account).

• Find the coupling conditions of the homogenized cytoplasm to the surrounding membranes.

Here we will only use the results. For the estimation of the effective diffusion coefficient, $D_{3,\text{eff}}$ for the homogenized cytoplasm, the numerical experiments with real membrane structures have already been performed [1,8]. Here we use effective diffusion coefficient from [17]

$$D_{3,\text{eff}} = 3.9517 \times 10^{-11}\ \text{m}^2\text{s}^{-1}$$

• **Sub-domain 4 (nuclear membrane)** Similar to sub-domain 2

$$\frac{\partial C_4}{\partial t} = \nabla.(D_4 \nabla C_4)\,,$$
$$\frac{\partial U_4}{\partial t} = \nabla.(D_4 \nabla U_4)\,.$$

• **Sub-domain 5 (nucleus)** In this domain, we have the following reaction system

$$C \xrightarrow{k_U} U\,,$$
$$C \xrightarrow{k_A} A\,.$$

The PDEs take the form

$$\frac{\partial C_5}{\partial t} = \nabla.(D_5 \nabla C_5) - (k_U + k_A) C_5\,,$$
$$\frac{\partial U_5}{\partial t} = \nabla.(D_5 \nabla C_5) + k_U C_5\,,$$
$$\frac{\partial A_5}{\partial t} = k_A C_5\,.$$

2.3 Interface Conditions

Since the species *C* and *U* must dissolve into the lipid (membrane) phase for the sake of transportation, at the interface between the different sub-domains, we need interface conditions. The conservation of mass leads to the continuity of flux between the different phases. The interface conditions for the concentration between the aqueous and membrane phases, are described by the dimensionless partition coefficient K_P. Hence the interface conditions at the interfaces take the form,

$$S_1 = K_{p,S} S_2 \qquad D_1 \frac{\partial S_1}{\partial \mathbf{n}_1} + D_2 \frac{\partial S_2}{\partial \mathbf{n}_2} = 0\,,$$
$$S_5 = K_{p,S} S_4 \qquad D_4 \frac{\partial S_4}{\partial \mathbf{n}_4} + D_5 \frac{\partial S_5}{\partial \mathbf{n}_5} = 0\,.$$

where $S = C, U$. Here $\mathbf{n}_i$ denotes the outward normal vector of the ith sub-domain. Also,

$$\mathbf{n}_1 = -\mathbf{n}_2 \text{ and } \mathbf{n}_4 = -\mathbf{n}_5$$

2.4 Boundary Conditions

We assume our system to be closed. So the domain consisting of extracellular part of cell is bounded. Hence, at the outer boundary, Neumann Boundary Conditions are needed. i.e.

$$\frac{\partial S_1}{\partial \mathbf{n}_1} = 0$$

Substances *B* and *A* are only restricted to the domains 3 and 5 respectively, so again we have the Neumann Boundary Conditions

$$\frac{\partial B_3}{\partial \mathbf{n}_3} = 0 \quad , \quad \frac{\partial A_5}{\partial \mathbf{n}_5} = 0\,.$$

2.5 Initial Conditions

We assume that, at initial time, only the concentration of *C*, is non-zero and having the value

$$C_1 = C_0|_{t=0}$$

where all other species have zero concentration at $t = 0$.

3. Experiments on Different Cell Architectures

Since we have already done the different experiments of varying cell geometry in [2], and we saw that, a change of the cellular architecture greatly affects the drug diffusion. Here in this paper, we have performed the different experiments not only for the comparison of results with the results of vitro experiments, but also to decide about the best possible shape of cell for the model which resembles the original cell in vitro.

In the first experiment, the cell is considered as spherical. The geometric constants (such as volume and thickness) were taken from [1], which has been described in Table 1.

Table 1: Geometric Constants

Constants	Value
Volume of one cell [m^3]	3×10^{-15}
Relative thickness of cell/nuclear membrane [m]	2×10^{-3}
Relative thickness of cytoplasm	3
Volume of cell medium [m^3]	10^{-5}
Membrane volume fraction in cell	0.25
Number of cells	2×10^{6}

In the remaining experiments, the cell is considered as non-spherical, having the shape of a fried egg sunny side up. The shape of cell was changed in these experiments keeping the geometrical constants (Table 1) same. For the following results the shape indicated in Figure 2 was used.

Figure 2. Diagram of quarter Part of an axi-symmetric cell

4. Detailed Modeling in Comsol Multiphysics

The model has been implemented in Comsol Multiphysics 3.5 [14] and Reaction Engineering Lab 1.5 [16]. This software uses the method of lines and the finite element method for the discretization with respect to the spacial independent variable.

In order to impose the interface conditions, we use a technique from the model library of the Chemical Engineering Module [15]. For example, at the interface between the extracellular and cellular membrane, the interface conditions can be replaced by

$$D_1 \frac{\partial S_1}{\partial \mathbf{n}_1} = M(S_2 - K_{p,S} S_1),$$

$$D_2 \frac{\partial S_2}{\partial \mathbf{n}_2} = M(K_{p,S} S_1 - S_2).$$

Where M is a (non-physical) very large constant. This penalty approach can be easily implemented in Comsol Multiphysics.

5. Simulation Results

In our simulations, constants and parameters from [1,17] have been used. These constants can be found in Table 2.

Table 2: Chemical Constants

Symbol	Constants	Value
D_1	Diffusion coefficient in cell/ nuclear membrane [m^2s^{-1}]	10^{-9}
D_2 ,D_4	Diffusion coefficient in extracellular water [m^2s^{-1}]	10^{-12}
D_5	Diffusion coefficient in nucleus [m^2s^{-1}]	2.5×10^{-10}
K_p	Partition coefficient	4.9×10^{-3}
k_U	Solvolytic reactivity forming U [s^{-1}]	7.7×10^{-3}
k_B	Catalytic efficiency [s^{-1}]	2.244
k_A	DNA adduct formation rate [s^{-1}]	6.2×10^{-3}

Simulations were performed for a time span of 600 sec. The comparisons between the results of concentrations of different species with respect to the time in the model with the actual results taken from the vitro experiments using mammalian cells [18] are shown in Figure 3, 4 and 5.

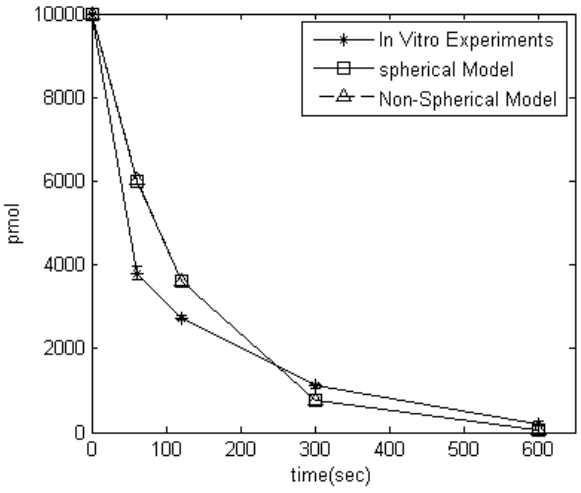

Figure 3. PAH Diol Epoxides (C in extracellular)

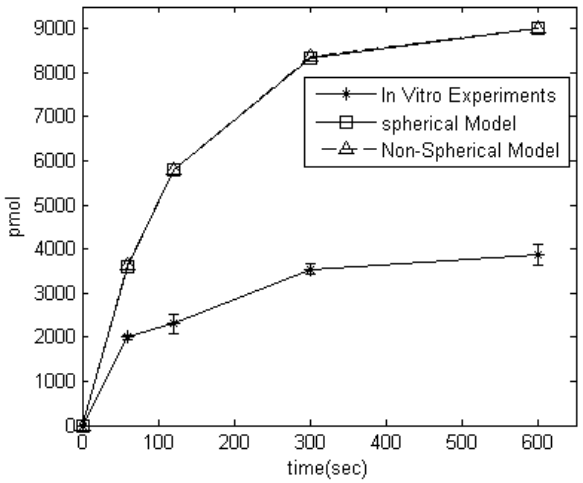

Figure 4. PAH Tetrol (U in extracellular)

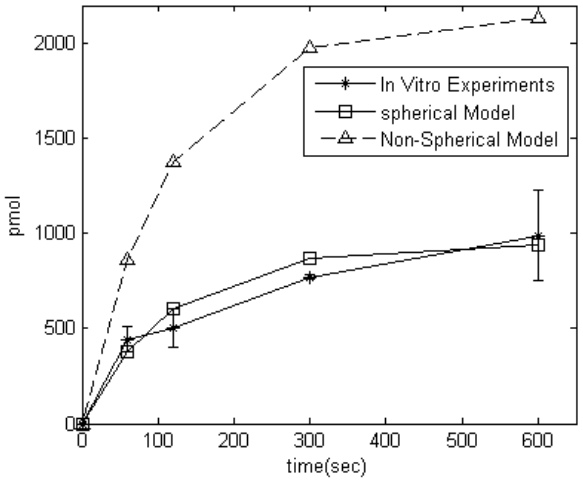

Figure 5. Glutathione Conjugate (B in cytoplasm)

In Figure 3, we see a very nice agreement of the results of in vitro experiments and the models in extracellular water. The large difference observed in Figure 4 can be explained in part by the fact that some reactions, e.g. protein binding, have not been considered. In Figure 5, there is a good agreement of the results of in vitro experiments and the spherical model, but there is a difference in the case of non spherical cell model. The reason is that, the shape of the cellular architecture has been considerably varied, which varies the concentration a lot [2].

Also the differences seen might be explained by the reason that the molecular dynamics within and outside the cell is much more complex than we assume in our model.

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