A Computational Model of Simulating Competitive Growth Factor Binding Under Flow *

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Abstract

A novel convection-diffusion-reaction model is developed to simulate fibroblast growth factor (FGF-2) binding to cell surface receptors (FGFRs) and heparan sulfate

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proteoglycans (HSPGs) under flow conditions within a cylindrical-shaped vessel or capillary. The model consists of (1) a set of coupled nonlinear partial differential equations (PDEs), the incompressible Navier-Stokes equations and the convection-diffusion transport equation, and (2) a set of coupled nonlinear ordinary differential equations (ODEs), the local binding and signaling by chemical kinetics. The time-dependent PDE system is discretized and solved by a second order implicit Euler scheme using the finite volume method. The ODE system is solved by a stiff ODE solver VODE using backward differencing formulation (BDF). The transient solution of FGF-2, FGFR, HSPG and their bound complexes for three different flow rates are computed and presented. Simulation results indicate that the model can predict the growth factor transport and binding to receptors with/without the existence of heparan sulfate in flow, as well as the effect of flow rate on growth factor-receptor binding. Findings from this study may provide useful information in understanding the impact of flow on growth factor binding and, ultimately, signaling in circulation.

**Key words:** Convection-diffusion-reaction, Fibroblast growth factor, Computer modeling, Heparan sulfate proteoglycans, Incompressible Flow.

1 Introduction

Basic fibroblast growth factor or fibroblast growth factor-2 (FGF-2) is a soluble protein which can stimulate the growth of many cell types including endothelial cells and some types of tumors [14, 19]. FGF-2, as well as other members of the FGF family bind to their tyrosine kinase receptors (FGFRs) as well as heparan sulfate proteoglycans (HSPGs) on cell surface. FGF-2 is found in extracellular matrices and circulation [1, 9, 13, 14, 18, 19], and, under normal conditions, the levels of FGF-2 found are relatively low [9]. These levels can, however, be substantially elevated in cases of disease and cancer [8, 28]. For example, high levels of FGF-2 in the circulation have been found in patients with angiogenic tumors [21]. The temporal and spatial distribution of FGF-2 is elemental to normal development as well as disease control [9] and can be regulated by HSPGs.
A quantitative model of convection-diffusion-reaction of FGF-2 in flow, however, has not yet been established. Dowd et al. [6] analyzed the transport of FGF-2 through Descemet’s membrane (DM), the basement membrane of the corneal endothelium. In their study, the diffusion of FGF-2 through the interstices of the membrane was considered, coupled with fast, reversible association of FGF-2 to resident heparan sulfate chains. Recently, Forsten et al. [16] published a more complete model about the kinetics of FGF-2 binding to heparin sulfate proteoglycans and MAP (mitogen-activated protein) kinase signaling. Their model suggests that FGF-2, its receptor, and a heparan sulfate proteoglycan interact simultaneously to form a high-affinity complex. The heparan sulfate chain could act by binding both FGF-2 and its receptor, facilitating the FGF-2-receptor interaction. In this manner, the cell surface proteoglycan and receptor could each bind FGF-2 and then come together to form a stable complex, as well as some compound dimers. The growth factor binding models published by Forsten and Fannon [9, 15, 16] were in a dish environment, in which the fluid flow and mass transport were not involved. A dish environment implies that the system geometry is planar, quite different from the current model in which a cylindrical tube is considered.

In many situations, the transport and binding of FGF-2 is closely related with circulation. This paper describes a general mathematical model and provides efficient numerical methods for simulating the binding of a ligand (FGF-2) simultaneously and competitively to two different binding sites, FGFR and HSPG, located on the surface of a capillary, under flow condition in vitro. In particular, we modeled FGF-2 transport and binding in a FiberCell bioreactor. The FiberCell bioreactor, shown in Fig. 1(a), is a typical reactor which allows the long-term culture and study of endothelial cells under flow [26]. The heart of the bioreactor system is a hollow fiber cartridge containing coated fibers on which cells can be cultured, and the flow rate can be adjusted by the system pump. The model for surface reactions that we have used were developed previously by Forsten-Williams and coworkers [15, 16]. As illustrated in Fig. 1(b), in the model, FGF-2 binds to FGFR and HSPG to form complexes of FGF-2-FGFR and FGF-2-HSPG, and the resulting complexes
may further bind to produce either dimers or FGF-2-FGFR-HSPG complexes, which may further bind to generate FGF-2-FGFR-HSPG dimers. The previous work using this model focused only on static culture conditions.

A coupled nonlinear convection-diffusion-reaction model for simulating heparan sulfate chain regulation over the growth factor binding under flow conditions is described here. To the best of our knowledge, this is the first complete computational model that combines fluid dynamics and kinetics of FGF-2. Within the model, we can simulate the dynamic environment of FGF-2 transport and quantitatively predict the impact of media flow on FGF-2 binding. This model provided the computational infrastructure needed to study the effects of heparan sulfate on growth factor-binding within a bioreactor system, to simulate the media flow system in capillaries in vitro, and to evaluate the effect of competing growth factor-binding proteins on cell surface under flow conditions. The model could serve as a prototype for the more advanced simulation of growth factor-binding to receptors as well as heparan sulfate chains in circulation in vivo. In particular, the model is used to predict the time-dependent distribution of FGF-2 in a capillary with complicated binding kinetics on the tube surface, where the flow velocity affects the concentration fields of FGF-2, FGFR, HSPG, and their bound complex.

The theoretical and experimental base of the convection-diffusion-reaction model of FGF-2 is as follows. Convection with media flow is controlled by flow velocity which can be obtained by solving the well established Navier-Stokes equations [11], diffusion in solution is determined by its diffusivity which can be estimated through theoretical analysis [12], and reaction kinetics are based on the model proposed by Forsten-Williams et al. [16]. The rest of the paper is arranged as follows. The complete mathematical model is described in Section 2, which includes the incompressible Navier-Stokes equations (Section 2.1), the mass transport equation (Section 2.2), and the competitive growth factor-binding mechanism (Section 2.3). Numerical schemes for solving the PDEs and ODEs are illustrated in Section 3. Numerical results are presented in Section 4 and discussed in Section 5. Finally a brief summary is given in Section 6.
2 Mathematical Modeling

Three types of physical processes are included in this coupled nonlinear model: the fluid flow, the transport of growth factor within the flow, and the chemical kinetics of the growth factors with cell surface proteins found on the capillary walls. These processes are simulated by incompressible Navier-Stokes equations for media flow, convection-diffusion transport equations for species conservation, and kinetic equations for molecular binding. The flow is considered independent of species transport and surface binding processes and is computed separately. After the flow field is obtained, we solve the transport equation, using the calculated velocity field. Since the molecular binding of FGF-2 to binding sites is assumed to take place on the tube surface only (i.e. to cell surface molecules), we may model the process as mass transport in a pipe with chemical reaction boundary conditions.

2.1 Incompressible Navier-Stokes equations

Our model was designed to simulate a straight cylindrical tube (i.e. a model capillary or bioreactor). A reasonable assumption for typical fluids used in vitro bioractor is that they are incompressible. Given our model design, it is convenient to write the governing equations in cylindrical coordinates. Assuming the flow is axisymmetrical and laminar, a 3D problem can be reduced to a 2D equivalent one. In cylindrical coordinates, all derivatives with respect to the circumferential direction are zero and the three velocity components are functions of the axial and radial directions only. If circumferential flow is not considered, the governing equations can be further simplified. The 2D time-dependent equations for mass and momentum in conservation form for incompressible flow in an axisymmetric coordinate system can be written as:

The mass conservation equation

\[
\frac{\partial u}{\partial r} + \frac{u}{r} + \frac{\partial v}{\partial x} = 0, \tag{1}
\]
the radial momentum equation

\[ \frac{\partial u}{\partial t} + \rho u \frac{\partial u}{\partial r} + \rho v \frac{\partial u}{\partial x} = \rho g_r - \frac{\partial p}{\partial r} + \frac{1}{r} \frac{\partial}{\partial r} \left( r \tau_{rr} \right) + \frac{\partial \tau_{rx}}{\partial x}, \]  

(2)

and the axial momentum equation

\[ \frac{\partial v}{\partial t} + \rho u \frac{\partial v}{\partial r} + \rho v \frac{\partial v}{\partial x} = \rho g_x - \frac{\partial p}{\partial x} + \frac{1}{r} \frac{\partial}{\partial r} \left( r \tau_{rx} \right) + \frac{\partial \tau_{xx}}{\partial x}. \]  

(3)

In Eqs. (1) ~ (3), \( \rho \) is the density, \( u \) is the radial velocity, \( v \) the axial velocity, \( p \) the dynamic pressure, \( g_r \) and \( g_x \) the radial and axial components of gravity respectively, and the stress tensors \( \tau_{rr}, \tau_{rx}, \) and \( \tau_{xx} \) are \( \tau_{rr} = 2\mu \frac{\partial u}{\partial r}, \tau_{rx} = \mu \left( \frac{\partial u}{\partial x} + \frac{\partial v}{\partial r} \right), \) and \( \tau_{xx} = 2\mu \frac{\partial v}{\partial x}. \)

For a Newtonian incompressible flow, the viscosity \( \mu \) is a constant.

### 2.2 Transport equation of FGF-2

The transport equation for FGF-2 in our model consists of two mechanisms, convection and dissipation. The convection term describes transport of local components along the streamlines of the flow, co-moving with the particles of the fluid. In our model, the velocity field of the flow is calculated separately and treated as a known. The dissipation term describes diffusive transport of components due to the gradient of FGF-2 concentration. The local concentration of a component changes with pressure difference, gravitational forces, and viscous dissipation. The mass of each species must be conserved. In the existence of chemical reaction, the coupling of mass transport and chemical kinetics in a circular pipe can be described by the following equations:

\[ \frac{\partial \phi_i}{\partial t} + \frac{1}{r} \frac{\partial (ru \phi_i)}{\partial r} + \frac{\partial (v \phi_i)}{\partial x} = \frac{1}{r} \frac{\partial}{\partial r} \left( K_r \frac{\partial \phi_i}{\partial r} \right) + \frac{\partial}{\partial x} \left( K_x \frac{\partial \phi_i}{\partial x} \right) + F_i(\phi_1 \ldots \phi_n), \quad 1 \leq i \leq n, \]  

(4)

where \( \phi_i \) is the concentration of species \( i \), \( u \) and \( v \) are the radial and longitudinal components of velocity, \( K_r \) and \( K_x \) the molecular diffusion coefficients, and \( F_i \) the rate of change due to kinetic transformations for each species \( i \). In the current model, only one
species, FGF-2, enters the capillary in the flow, so here $\phi_i$ is simply FGF-2. Molecular
binding happens only on the tube surface, that is to say $F_i$ is valid merely on the pipe
surface. The reactants and products involved in the chemical kinetics include FGF-2,
FGFR, HSPG, FGF-FGFR complex and its dimer, FGF-HSPG complex and its dimer,
FGF-HSPG-FGFR complex and its dimer, with a total of nine species ($n = 9$). The
detailed chemical kinetics of these species will be discussed in Section 4. The boundary
conditions of Eq. (4) are

$$\frac{\partial \phi_i}{\partial r} = f_i(t, x, C_i) \quad \text{at} \quad r = R, \quad \frac{\partial \phi_i}{\partial r} = 0 \quad \text{at} \quad r = 0.$$  \hspace{1cm} (5)

The first boundary condition accounts for interactions at the vessel wall ($R$ is the radius
of the capillary and $f_i$ the rate of binding to cells), while the second boundary condition
reflects the axial symmetry.

### 2.3 Competitive binding kinetics

The change of concentration with respect to time for various species may be described by
a set of ordinary differential equations (ODEs) in terms of mole or mass fraction. FGF-2
binding involves a series of molecular activities, including binding to receptors, HSPG,
and some intermediate complexes. Previous models have been proposed to investigate
the role of low-affinity receptors on high-affinity receptor binding [8, 15, 16]. As was done
previously [15, 16], we assumed that each cell has a homogeneous distribution of FGFR and
HSPG binding sites [15]. Synthesis of receptors and surface HSPG as well as internalization
of unbound receptors, surface HSPG, and FGF-2 complexes are considered as well. There
are eight chemical reactions in the model and nine species are involved (See Appendix for
equation and Table 1 for reactions and parameters). A directed graph is used to represent
the reaction network of molecular binding in the base model, as shown in Fig. 1(c). In
the directed graph, the species involved in the reactions are represented by vertices, and
the reactions are represented by directed edges. The number of edges, however, is not

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necessarily equal to the number of reactions, since there may be more than one reactants and/or products in a single reaction. The internalization of species is not included in the directed graph but is included in the model. In Fig. 1(c), single arrowheads indicate irreversible reactions and double arrowheads indicate reversible reactions. For example, P and L generate G, and G decompose to produce P and L; G and R generate T, but T does not decompose to produce G and R.

Using FGF-2-HSPG as an example, the change of FGF-2-HSPG with time is described as

\[
\frac{dG}{dt} = k_f^P LP - k_f^P G - k_c RG - k_c G^2 + 2k_{int}G_2 - k_{int}G,
\]

where \(k_f^P LP\) is the production of FGF-2-HSPG due to the binding of FGF-2 to unbound HSPG, \(-k_f^P G\) is the dissociation of FGF-2-HSPG to FGF-2 and HSPG, \(-k_c RG\) is the loss due to surface coupling of an FGF-2-HSPG complex with unbound FGFR, \(-k_c G^2\) is the loss due to the formation of FGF-2-HSPG dimers, \(2k_{int}G_2\) is the increase due to the dissociation of FGF-2-HSPG dimers, and \(-k_{int}G\) is the loss due to internalization. The remaining eight nonlinear ordinary differential equations can be found in the Appendix.

3 Numerical Schemes

Finite volume method [27, 23] has been widely used in solving both steady and unsteady fluid mechanics problems, for it is easy to implement and guarantees flux conservation. For our model, cell-centered finite volume approach was applied to discretize the partial differential equations. The advantage of a cell-centered arrangement is that second order accuracy is achieved, since the nodal value represents the mean over the control volume and the node is located at the centroid to the control volume. The Navier-Stokes equations consist of a set of coupled nonlinear partial differential equations, the solution of which is not a trivial task, and we adopt the SIMPLER algorithm [11, 27] in the current computation. To solve the Navier-Stokes equations, two loops are required. The inner iteration handles each of the individual equations of momentum and energy, while the
Table 1: Chemical reactions included in the base model using mass-action kinetics and their relevant parameters [16]. The concentration of species on the tube surface can be converted from 1#/cell to $4.82 \times 10^{-14}$ µmol/cm².

<table>
<thead>
<tr>
<th>Chemical reaction</th>
<th>Kinetic formula</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L + R \xrightleftharpoons{k^R_f}{k^P_f} C$</td>
<td>$k^R_f [R][L], k^P_f [C]$</td>
<td>$k^R_f = 2.5 \times 10^8$ M⁻¹ min⁻¹, $k^P_f = 0.048$ min⁻¹</td>
</tr>
<tr>
<td>$L + P \xrightleftharpoons{k^P_f}{k^P_c} G$</td>
<td>$k^P_f [P][L], k^P_c [C]$</td>
<td>$k^P_f = 0.9 \times 10^8$ M⁻¹ min⁻¹, $k^P_c = 0.068$ min⁻¹</td>
</tr>
<tr>
<td>$R + G \rightarrow T$</td>
<td>$k_c [R][G]$</td>
<td>$k_c = 0.001$ min⁻¹ (#/cell)⁻¹</td>
</tr>
<tr>
<td>$C + P \xrightarrow{k_c} T$</td>
<td>$k_c [R][G]$</td>
<td>$k_c = 0.001$ min⁻¹ (#/cell)⁻¹</td>
</tr>
<tr>
<td>$T \xrightarrow{k_c^L} L + R + P$</td>
<td>$k_c [R][G]$</td>
<td>$k^L_c = 0.001$ min⁻¹</td>
</tr>
<tr>
<td>$C + C \xrightarrow{k_{uc}} C_2$</td>
<td>$k_c [C]^2$</td>
<td>$k_c = 0.001$ min⁻¹ (#/cell)⁻¹, $k_{uc} = 1$ min⁻¹</td>
</tr>
<tr>
<td>$G + G \xrightarrow{k_{uc}} G_2$</td>
<td>$k_c [G]^2$</td>
<td>$k_c = 0.001$ min⁻¹ (#/cell)⁻¹, $k_{uc} = 1$ min⁻¹</td>
</tr>
<tr>
<td>$T + T \xrightarrow{k_{uc}} T_2$</td>
<td>$k_c [T]^2$</td>
<td>$k_c = 0.001$ min⁻¹ (#/cell)⁻¹, $k_{uc} = 1$ min⁻¹</td>
</tr>
<tr>
<td>$R \xrightarrow{k_{int}} R_{int}; P \xrightarrow{k_{int}} P_{int}$</td>
<td>$k_{int} [R]; k_{int} [P]$</td>
<td>$k_{int} = 0.005$ min⁻¹</td>
</tr>
<tr>
<td>$C \xrightarrow{k_{int}} C_{int}; G \xrightarrow{k_{int}} G_{int}$</td>
<td>$k_{int} [C]; k_{int} [G]$</td>
<td>$k_{int} = 0.005$ min⁻¹</td>
</tr>
<tr>
<td>$T \xrightarrow{k_{int}} T_{int}; C_2 \xrightarrow{k_{int}} C_{2_{int}}$</td>
<td>$k_{int} [T]; k_{int} [C_2]$</td>
<td>$k_{int} = 0.005$ min⁻¹, $k_{int} = 0.078$ min⁻¹</td>
</tr>
<tr>
<td>$G_2 \xrightarrow{k_{int}} G_{2_{int}}; T_2 \xrightarrow{k_{int}} T_{2_{int}}$</td>
<td>$k_{int} [G_2]; k_{int} [T_2]$</td>
<td>$k_{int} = 0.005$ min⁻¹, $k_{int} = 0.078$ min⁻¹</td>
</tr>
</tbody>
</table>
outer iteration deals with the coupling and nonlinearity. For unsteady flow using implicit discretization, the discretized linear equations need not be solved very accurately at each outer iteration. Usually a few iterations of a linear solver is enough. More accurate solution will not reduce the number of outer iterations but may increase the computing time [11].

A transient solution was pursued in the current simulation. To achieve higher order time accuracy, we used a quadratic backward approximation for the time derivative term. Such arrangement gives us second order time accuracy [11]. In the transport equation, the convective term needs special treatments for stability consideration [23]. A frequently used technique is upwind differencing. However, upwind discretization provides only first order spatial accuracy. For the sake of numerical stability as well as higher order spatial accuracy, a deferred correction numerical strategy was used here [11], which is a combination of the first order upwind differencing and the second order central differencing. The diffusive terms are discretized by central difference. Due to implicit discretization of the governing equations, a linear system is formed and needs to be solved efficiently at each time step.

On the tube surface, a system of simultaneous ordinary differential equations was obtained from the set of biochemical reaction equations, in the form of mass action kinetics. Let vector $\mathbf{y}$ be the species populations in the chemical reaction networks, $\mathbf{y} = [y_1 \ y_2 \ \cdots \ y_n]^T$, the vector notation of the system of ordinary differential equations may be written as

$$\frac{d\mathbf{y}}{dt} = \mathbf{f}(\mathbf{y}, t).$$

(7)

For the coupled nonlinear ODE system with time $t$ as the sole independent variable, our initial attempt using a fourth-order standard Runge-Kutta method was not successful due to the stiffness of the system. We then turn to a stiff ordinary differential equation solver VODE using the backward differentiation formulation [2].

Using backward differencing formulation (BDF), Eq. (7) is solved by finite difference
as
\[ y^n = y^{n-1} + \Delta t f(y^n). \] (8)

If all equations are linear, we may replace \( f(y) \) with \( f(y) = Ay \), where \( A \) is the coefficient matrix, and we can use a matrix vector product to find the rate of change of the concentration of relevant species due to a series of reactions, and the ordinary differential equations can be solved directly. The solution of the linear system is then found to be

\[ y^n = (I - \Delta t A)^{-1} y^{n-1}, \] (9)

where \( I \) is the identity matrix.

For a nonlinear system of equations, the solution of Eq. (8) is more complicated. Newton's method is frequently used to solve nonlinear systems, and the solution of the nonlinear system is

\[ y^n = y^{n-1} + \Delta t (I - \Delta t J)^{-1} y^{n-1}, \] (10)

where \( J \) is the Jacobian matrix, which is calculated as \( J = \frac{\partial f}{\partial y} \) to linearize the system. Since the Jacobian matrix \( J \) is generally not constant, at each step, we need to evaluate matrix \( J \) and compute the inverse of the matrix \( I - \Delta t J \). The Jacobian matrix can be evaluated either analytically or numerically. Analytical Jacobian is always preferred, since the computation of analytical Jacobian matrix is both numerically more accurate and computational more efficient. The nonlinear system of ordinary differential equations is solved by the VODE solver [2], which can handle both stiff and nonstiff systems. VODE uses a variable-coefficient form of BDF method. In the stiff case, the Newton iteration is relaxed by a scalar factor. VODE provides a flexible application programming interface (API) through two external subroutines, FEX and JEX, where FEX allows users to provide a set of rate equations from chemical kinetics, the right-hand side vector \( f(y, t) \), and JEX allows users to calculate analytical Jacobian matrix. Users also have to specify initial values of the system, as well as some parameters, such as relative and absolute tolerances, the lengths of real work array and integer work array. In the current computation, the
relative tolerance is set as $10^{-4}$, and the absolute tolerance is species dependent, i.e., each species may have its own absolute tolerance. (We used BDF method with user-supplied full Jacobian.)

4 Results

4.1 Media flow in capillary and model validation

We use the FiberCell bioreactor as a motivation model to simulate FGF-2 transport and binding in circulation. The parameters of the FiberCell bioreactor are as follows, the radius of an individual fiber $R = 0.35$ mm, the fiber length $L = 10$ cm, the number of tubes or fibers per cartridge $N = 20$, the flow rate is adjustable in the range of $r = 80 \sim 0.5$ ml/min. The viscosity is taken as $\mu = 0.04$ dyne·s/cm$^2$, and the density of the fluid is taken as $\rho = 1060$ kg/m$^3$ [26]. The FiberCell bioreactor can be used to simulate capillary flows by adjusting the flow rate to around $0.5 \sim 5.0$ ml/min. It was reported by Tooke and Milligan [31] that the velocity of capillary blood flow in the human arterial limb was around 1 mm/s, which could be lower for some patients.

The numerical solution of medium flow is obtained by the 2D incompressible Navier-Stokes equations. Given the velocity, dimension and medium properties, the Reynold number is less than 1, the flow in capillary is essentially laminar. The boundary conditions for the flow simulation are, a uniform flow velocity of $u = u_0$ at the tube entrance, non-slip boundary condition on tube surface $u = v = 0$, symmetric boundary condition along the tube centerline $v = 0$ and $\frac{\partial u}{\partial r} = 0$, and zero velocity gradient at flow exit. The velocity distribution in the artificial capillary is very well predicted by the current incompressible code. This claim is supported by a comparison of $u$ velocity profile in the fully-developed region between the numerical and analytical solutions, shown in Fig. 2. For a fully-developed laminar flow in a circular pipe, the velocity profile can be found analytically as $u = u_{max} \left(1.0 - \frac{r^2}{R^2}\right)$, where $u_{max} = 2.0 \times u_{inlet}$. The steady-state flow field in two-dimension is presented in [30].
The accuracy of the numerical solution depends very much on the mesh size. Ideally, the numerical solution should be very close to the exact solution of the PDEs in a very fine mesh. However, due to the limited computing resources and the fact that a too small grid spacing may cause unexpected numerical difficulties, using an extremely fine mesh may not be an optimal strategy. A practical way of achieving satisfactory numerical accuracy is to find a so-called grid-independent solution by the method of trial and error, in which we refine the mesh continually until the results between two consecutive trials are very close. Fig. 3 shows the dependency of numerical solution on mesh size in terms of FGF-2 concentration on the capillary surface. To be specific, three types of mesh size were used, 1200 × 20, 1400 × 24, and 1600 × 24, where the number of control volumes in the axial direction is taken as 1200, 1400, and 1600, and the number of control volumes in the radial direction is taken as 20 and 24, respectively. Figs. 3(a) to 3(d) are the concentration distribution of FGF-2 along the capillary surface at the time of 5, 10, 20, and 40 minutes, where triangles represent the results of mesh size 1200 × 20, solid line represents the results of mesh size 1400 × 24, and circles represent those of mesh size 1600 × 24. It is clear that the numerical solutions for the two cases of 1400 × 24 and 1600 × 24 are close enough to be considered as grid-independent. Therefore, in the rest of the chapter, all numerical results are presented using a mesh size of 1400 × 24.

4.2 Ligand transport and binding in the absence of HSPG

For our simulation, the transport equation was solved after the flow velocity was obtained by solving the Navier-Stokes equations. The boundary conditions applied to the convection-diffusion equation, Eq. 4, were as follows. At the tube entrance, a uniform concentration of $\phi = L_0 = 0.0556$ nM is prescribed. At the centerline of the capillary, a symmetric boundary condition $\frac{\partial \phi}{\partial r} = 0$ was used. At the outlet, we extrapolated the concentration of ligand by zero gradient $\frac{\partial \phi}{\partial r} = 0$. At the tube wall, a reaction boundary condition was implemented to reflect the growth factor binding. On the tube surface, the
conservation equation for the growth factor FGF-2 can be written as

$$\frac{\partial \phi}{\partial t} + \mathbf{n} \cdot \mathbf{|F|} = G \quad (11)$$

where $\phi$ is the concentration of FGF-2, $\mathbf{|F|}$ denotes the diffusion flux through tube surface, $\mathbf{n}$ is the unit vector normal to the surface and pointing from liquid to tube surface, and $G$ is the surface differentiation rate due to biochemical reaction. At any moment, the diffusion flux has to be balanced by chemical reaction, i.e., $\frac{\partial \phi}{\partial t} = 0$. Similar to reference [12], the boundary condition for FGF-2 transport can be further expressed as

$$D \frac{\partial \phi}{\partial r} |_{r=R} = \left( -k^R C + k^T T - k^P P - k^R G \right) /CONST, \quad (12)$$

where, $D$ is the diffusivity taken as $9.2 \times 10^{-7}$ cm$^2$/s estimated from the Stokes-Einstein equation [12], $k^R$, $k^T$, $k^P$, $k^R$, and $k^R$ are parameters listed in Table 1, and $CONST$ is used for unit conversion, to convert the diffusion flux in unit of mol/(L·s) to surface differential rate due to chemical reaction in unit of $(\text{#/cell · min})$. According to Elhadji et al. [7], bovine aortic endothelial cells could be cultured and seated on the surface of a capillary in a cartridge of a bioreactor with a surface concentration of $2.9 \times 10^4$ cells/cm$^2$. Based on the concentration, we can establish a unit conversion from (number of molecules)/cell to $\mu$mol/cm$^2$, i.e., 1 (number of molecules)/cell is equivalent to $4.82 \times 10^{-14}$ $\mu$mol/cm$^2$.

To validate the mathematical model and software, we eliminated both the convection and diffusion parts of the convection-diffusion-reaction model, and were able to reproduce the theoretical predictions with respect to the time dependent concentration distribution of the model involving nine species in the time interval of 0 ~ 100 minutes, given by Forsten-Williams et al. [16], who obtained the results using Matlab. Then we included the functions of convection and diffusion and verified conservation of mass for the FGFR and HSPGs in the absence of synthesis and internalization/degradation.

Let us first simulate FGF-2 transport in capillary without surface binding. The solution presented in the paper is based on a mesh size of $1400 \times 24$, and it is grid independent.
The computation results of FGF-2 concentration has been scaled with respect to the uniformly specified inlet boundary concentration, \( L' = L/L_0 \), where \( L_0 = 0.0556 \text{ mol/L} \). The uniform inlet flow velocity is set as \( u_0 = 0.1732 \text{ mm/s} \). Initially, there is no ligand in the solution. To start with, at \( t = 0 \), we enforce the boundary condition of \( L = L_0 \) at the tube entrance. Ligand has been transported into the flow by convection and diffusion. The time-dependent solution of FGF-2 concentration at \( t = 5, 10, 20, \) and 40 minutes is obtained. The FGF-2 concentration on the capillary surface has been shown in Figs. 4(a) \( \sim \) 4(d). At \( t = 5 \) minutes, ligand is observed in only a small portion of the tube. At \( t = 10 \) minutes, ligand occupies a large portion of the tube. At \( t = 20 \) minutes, ligand can be seen at tube exit. The visualization of FGF-2 concentration in the capillary can be found in [30]. As expected, without surface binding, the concentration of FGF-2 on the capillary surface is the same as that in the solution, and reaches a constant when the time is long enough.

Next, we examine FGF-2 and receptors binding. Assuming that receptors are attached on the capillary surface, FGF-2 molecules move from solution to capillary surface to bind the receptors. This is the initial value problem, which is modeled by assigning an initial condition of receptor concentration of \( R_0 = 1.6 \times 10^4 \text{ #/cell} \) on the capillary surface. We present the concentration distribution of free FGF-2 in Figs. 4(a) \( \sim \) 4(d) after FGF-2 and receptors binding. The figures clearly show the depletion of FGF-2 as a result of binding to receptors. Due to biochemical reactions on the tube surface, higher ligand concentration is observed in the tube center than near tube surface, and the details have been explained in reference [30].

Fibroblast growth factors bind to their receptors to form the new FGF-2-FGFR complexes, which further bind to form FGF-2-FGFR dimers. It is assumed that both FGF-2-FGFR complexes and their dimers are also located on the capillary surface. Figs. 5(a) and 5(b) exhibit the dependency of FGF-2-FGFR and their dimers on location and time. Five time spans are considered here, \( t = 5, 10, 20, 40, \) and 60 minutes. The model correctly predicts the dependency of FGF-2-FGFR concentration on time and location.
For the cases of 5 and 10 minutes, FGF-2-FGFR complexes are seen in a part of the capillary. After 20 minutes, FGF-2-FGFR complexes can be seen in the whole capillary. The longer time we run the model, the higher concentration of FGF-2-FGFR we observe. By comparing the concentration distribution of FGF-2-FGFR and its dimer for the cases of 5 and 10 minutes (Figs. 5(a) and 5(b)), we can see that the model predicts that the formation of FGF-2-FGFR dimer is after the formation of FGF-2-FGFR.

### 4.3 Effect of HSPG on ligand transport and binding

Heparan sulfate proteoglycans (HSPG) are macromolecules composed of linear heparan sulfate chains attached to a protein core [3, 5]. FGF-2 can bind to specific HSPG sites with relatively high affinity [4]. To determine the impact of HSPG on FGF-2 transport and receptor binding, we included HSPG on the capillary surface with an initial concentration of \( P_0 = 1.6 \times 10^6 \text{#/cell} \). The concentration of FGF-2 on capillary surface is displayed in Fig. 6(a) for the time spans of 5, 10, 20, 40, and 60 minutes. It is clear that the FGF-2 concentration is reduced significantly after adding HSPG with a concentration of \( P_0 \), by comparing the results in Fig. 4 and Fig. 6(a). The reduction of FGF-2 concentration is due to ligand and HSPG binding to form FGF-2-HSPG complexes.

We further investigate how the HSPG concentration affects FGF-2 and receptor binding. Seven cases are studied, which correspond to the following concentrations 0, \( 1.6 \times 10^4 \), \( 1.6 \times 10^5 \), \( 2.0 \times 10^5 \), \( 4.0 \times 10^5 \), \( 8.0 \times 10^5 \), and \( 1.6 \times 10^6 \) (#/cell) respectively. The results are plotted in Fig. 6(b) in terms of the concentration of free receptors for the time of 60 minutes for the first five cases. The results for cases 6 and 7 (\( 8.0 \times 10^5 \) and \( 1.6 \times 10^6 \) #/cell) are not shown in Fig. 6(b), since the resulting concentration of free receptors is less than 5 #/cell for the both cases, and they are overlapped with case 5 when plotted in the same figure. The concentration of free receptor is decreased after adding HSPG, which is largely due to the binding between FGF-2-HSPG and FGFR to form FGF-2-FGFR-HSPG complexes and their dimers. Almost no free receptors can be found on the capillary surface when the HSPG concentration is increased to \( 4.0 \times 10^5 \) (#/cell)
and above, according to the model prediction. This may be different from observations in a dish environment, where the concentration of FGF-2 is considerably low due to a bolus input of FGF-2. In our case, we assume a constant supply of FGF-2 at the tube entrance, so the concentration of FGF-2 in the solution is much higher. As an outcome of high FGF-2 concentration, a significant binding between FGF-2 and HSPG is still possible even though the concentration of HSPG is very low. The model also predicts that the concentrations of FGF-2-FGFR and its dimer are reduced by adding HSPG (results not shown). This is due to the high affinity binding of FGF-2-FGFR and HSPG to form FGF-2-FGFR-HSPG complexes. The dependency of FGFR and its binding complexes on HSPG concentration is not linear. For example, the concentration change of FGFR due to an increase of HSPG from 0 to $1.6 \times 10^4$ #/cell is not as significant as that of FGFR when the concentration of HSPG is increased from $1.6 \times 10^4$ to $1.6 \times 10^5$ #/cell. This observation may suggest that FGF-2 and receptor binding can be effectively regulated by carefully adjusting HSPG concentration.

4.4 Effect of flow on ligand transport

Based on our proposed convection-diffusion-reaction model and the corresponding simulation software, the effect of bio-fluid flow on ligand transport, is able to be investigated systematically. Figs. 7, 8 and 9 are the time dependent concentration distribution of ligand in the capillary of flow velocities $u = 10.4$ mm/min, $u = 5.2$ mm/min, and $u = 2.08$ mm/min respectively. All these three figures display the transient concentration field of FGF-2 in capillary for the first hour as FGF-2 is added to the tube inlet. Five subfigures are included in each individual of Figs. 7, 8, and 9, for different time intervals, $t = 5$, $t = 10$, $t = 20$, $t = 40$, and $t = 60$ minutes. Our results clearly indicate that FGF-2 distribution is greatly affected by flow velocity. An increased flow velocity results in high concentrations of FGF-2 on the capillary surface and at the tube exit, shown in Fig. 7. On the contrary, a reduced velocity lowers concentrations of FGF-2 on the capillary surface as well as tube exit, which can be seen in Fig. 9. If we denote high velocity $u = 10.4$
mm/min, middle velocity \( u = 5.2 \) mm/min, and low velocity \( u = 2.08 \) mm/min in the three test cases, for example, at \( t = 5 \) minutes, FGF-2 occupies more than a half space of the capillary in high velocity case (Fig. 7(a)), reaches about one fourth of the tube space in middle velocity case (Fig. 8(a)), and propagates to a location less than one eighth of the tube length in the low velocity case (Fig. 9(a)). At \( t = 60 \) minutes, the normalized concentration of FGF-2 at tube exit is between 0.6 and 0.7 in high velocity case, around 0.4 in middle velocity case, and less than 0.1 in the low velocity case.

5 Discussion

Growth factors play a very important role in modulating cell activities of proliferation and differentiation. The MAP kinase pathway starts with growth factor ligand binding to its transmembrane receptors followed by the activation of tyrosine kinases inherent in the receptor molecules [16, 29]. Such activation triggers the dimerization and phosphorylation of the transmembrane receptors. Computational models have recently been applied to growth factor signaling systems, most of which are epidermal growth factor (EGF) systems [24, 25]. A complex model has been used by Schoebert et al. [29] to study the activation of the MAP kinase signaling pathway by EGF, and intracellular signaling steps have been represented by a majority of the 94 compounds involved in the model. Complex models with up to 42 reactions have been developed by Kholodenko and collaborators [22] to interpret EGF signaling by considering downstream interconnections. In contrast, far less modeling work has been done on FGF system. Our work focuses on the modeling of extracellular binding of FGF-2 on cell surface. Forsten et al. [15] and Fannon et al. [10] have previously studied the regulation of heparin and heparin-like molecules on FGF-2 binding in solution. Filion and Popel [12] proposed a model to address the effect of FGF-2 dimerization on surface interactions. Forsten-Williams et al. more recently proposed a kinetic model [16], which consists of 60 reactions and 31 components including FGFR and HSPG dimerization, to investigate FGF-2 binding to heparan sulfate proteoglycans and MAP kinase signaling. That model is in a static environment where the concentrations
of receptor ligand, receptors, HSPGs, complexes, dimers and triads are assumed to be uniformly distributed in the solution. This assumption is away from in vivo situation to some extent. This paper presents a dynamic model for FGF-2 binding and signaling on cell surface, and the concentrations of species are not necessarily assumed to be uniformly distributed in space. Currently we adopt the FGF-2 surface binding model proposed by Forsten et al. [16], in which dimers of FGFR or HSPG are included as important signaling compounds. This model further assumes that the first step in high affinity dimerization is the formation of FGF-2-FGFR-HSPG triad and triads are formed by FGF-2 binding to FGFR or HSPG. The significance of dimers in signaling can be seen from their concentrations. For example, according to our computation results, the concentrations of HSPG dimers and FGF-2-FGFR-HSPG dimers are in the order of $10^3$, which is too high to be neglected.

The binding kinetics and signaling pathways of FGF-2 are very complicated, and many mathematical models have been proposed to predict the behavior of FGF-2 on cell surface binding [6, 9, 12, 16, 17, 24]. These models include pure reaction models [9, 16, 17], where a system of ordinary differential equations is provided and it is assumed that the movement of FGF-2 is not considered, and reaction-diffusion models [6, 12, 25], which are relatively more complex and the movement of FGF-2 molecules from fluid to cell surface is modeled by diffusion. Filion and Popel [12] proposed a one-dimensional diffusion-reaction model, in which FGF-2, FGF-2 dimers, soluble heparin-like glycosaminoglycans (HLGAGs), FGF-2-HLGAG compounds, and FGF-2-dimer-HLGAG compounds are located in the fluid layer. Those molecules move from fluid to cell surface by diffusion. The diffusion model is valid only if the fluid is quiescent, which is not consistent with the actual biological environment of moving bio-fluids. Our model is unique, in which a coupled convection-diffusion-reaction model is applied, and the motion of bio-fluids is fully considered.

Our dynamic model puts FGF-2 binding and signaling in flow environment of a capillary, where FGF-2 molecules move with circulation flow inside capillary, bind to receptor
and co-receptor molecules on tube surface, and signal MAP kinase pathway. Under current computation frame work, the Reynolds number is far less than the critical value for transition to turbulence, and the capillary flow is typical Hagen-Poiseuille flow with parabolic profile, as indicated by our simulation results. The molecular motion of FGF-2 is represented by a convection-diffusion equation in macroscale in terms of concentration distribution, which is determined by media flow, diffusion coefficient of FGF-2 in media, and the rate of change of FGF-2 due to reaction. Since the reaction rate of FGF-2 is obtained by the coupled differential system, the FGF-2 distribution in flow and the surface concentrations of receptors, complexes, dimers and triads are actually coupled. Our numerical solution reveals that the concentrations of FGF-2, FGFR, HSPG, FGF-2-FGFR, FGF-2-HSPG, FGF-2-FGFR-HSPG, FGF-2-FGFR dimer, FGF-2-HSPG dimer, and FGF-2-FGFR-HSPG dimer are indeed location dependent, as can be seen in Figs. 4, 5, 6, 7, 8, and 9 and reference [30]. No direct results can be found in literature for simulating FGF-2 binding and signaling using a convection-diffusion-reaction model. Both experimental and computational work is required to investigate FGF-2 binding and signaling under flow condition, and our results may serve as a useful starting point.

In many cases, cell surface interactions include growth factor binding to more than a single receptor type. For example, heparin binding fibroblast growth factor-2 binds to heparan sulfate proteoglycans on the cell surface and within extracellular matrix. In this scenario, HSPG molecules have been considered accessory co-receptors serving to facilitate tyrosine kinase receptor binding [16]. Recent investigations indicate that HSPG molecules can act independently as signaling molecules besides their role as receptor binding partner [5, 16, 20]. Forsten-Williams et al. found in their paper [16] that HSPG played an important role in the kinetics of FGF-2 binding to FGFR and in the absence of FGFR signaling the kinetics of HSPG binding and dimerization correlated well with Erk1/2 signaling, indicating intracellular signal generated by FGF-2 binding to HSPG, an alternative receptor type.
6 Summary

A novel computational model is developed as a tool to reveal the dynamic interactions among growth factors and their cell surface receptors under flow. We have applied this computational model to the study of FGF-2, its receptors, and HSPGs. The complex model is solved by efficient PDE and ODE solvers. To be specific, our convection-diffusion-reaction model provides a new way to simulate FGF-2 binding and signaling on cell surface. A single proteoglycan species is considered in the current simulation, but additional proteoglycan species could be included in the model without much effort. Our results indicate that the concentration of HSPG has a significant impact on the populations of binding sites and thereby formed complexes on the cell surface, as well as FGF-2 concentration within the media. Numerical results shown in the paper clearly demonstrate the significance of flow condition on FGF-2 transport, binding and signaling. Additional experimental and computational studies are needed for a better understanding of how cellular proteoglycans impact growth factor binding and signaling in vivo environment to achieve the goal of disease-healing by manipulating FGF-2-mediated cell activity.

Appendix

The model system used in the current study consists of the following 9 equations, which describe the rate of components change with respect to time. This model is about the system that expresses only a single class of HSPG [16]:

\[
\frac{dR}{dt} = -k_f^R L R + k_r^R C + k_r^T T - k_c^R G - k_{int}^R R + V_R, 
\]

(13)

\[
\frac{dC}{dt} = k_f^R L R - k_r^R C - k_c C P - k_c C^2 + 2k_{uc} C_2 - k_{int} C, 
\]

(14)

\[
\frac{dC_2}{dt} = \frac{k_c}{2} C^2 - k_{uc} C_2 - k_{int}^D C_2, 
\]

(15)
\[
\frac{dT}{dt} = k_c R G + K_c C P - k_r^T T - k_c T^2 + 2k_{uc} T_2 - k_{int} T,  
\]
\[
\frac{dT_2}{dt} = \frac{k_c}{2} T^2 - k_{uc} T_2 - \frac{k_{int}}{2} T_2,  
\]
\[
\frac{dP}{dt} = -k_f^P L P + k_r^G G + k_r^T T - k_c C P - k_{int} P + V_P,  
\]
\[
\frac{dG}{dt} = k_f^P L P - k_r^P G - k_c R G - k_c G^2 + 2k_{uc} G_2 - k_{int} G,  
\]
\[
\frac{dG_2}{dt} = \frac{k_c}{2} G^2 - k_{uc} G_2 - k_{int} G_2,  
\]
\[
V \frac{dL}{dt} = -k_f^R L R + k_r^R C + k_r^T T - k_f^P L P + k_r^P G  
\]

where \( L \) is FGF-2, \( R \) is FGFR, \( C \) is FGF-2-FGFR complex, \( P \) is HSPG, \( G \) is FGF-2-HSPG complex, \( T \) is FGF-2-HSPG-FGFR complex, \( C_2 \) is FGF-2-FGFR dimer, \( G_2 \) is FGF-2-HSPG dimer, and \( T_2 \) is FGF-2-HSPG-FGFR dimer.

References


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Figure 1: Sketch of the mathematical model. The symbols in the sketch are as follows: L=FGF-2, R=FGFR, P=HSPG, C = FGF-2-FGFR complex, G=FGF-2-HSPG complex, C2=FGF-2-FGFR dimer, G2=FGF-2-HSPG dimer, T=FGF-2-FGFR-HSPG complex, and T2=FGF-2-FGFR-HSPG dimer. (a) Schematic diagram of a FiberCell bioreactor (b) Growth factor binding to receptors and HSPG and the formation of various compounds on the surface of a capillary. (c) The reaction pathway for the base model.
Figure 2: Comparison of velocity profile of fully-developed laminar flow in a circular pipe between numerical and exact solutions. The numerical results were obtained at $x = 5.0$ cm
Figure 3: The dependency of numerical solution on mesh size in the case of surface reaction at the temperature of 4 °C, (a) at $t = 5$ minutes, (b) at $t = 10$ minutes, (c) at $t = 20$ minutes, (d) at $t = 40$ minutes, and (e) at $t = 60$ minutes, where triangles, solid line, and circles represent numerical results of mesh size 1200 × 20, 1400 × 24, and 1600 × 24 respectively. In the figure, $x$ is the direction down the tube and $[L]/[L_0]$ is the scaled FGF-2 concentration with $L_0$ the concentration at the entrance.
Figure 4: FGF-2 concentration on capillary surface in the cases of no surface binding and binding to its receptor only at 4 °C, (a) at $t = 5$ minutes, (b) at $t = 10$ minutes, (c) at $t = 20$ minutes, and (d) at $t = 40$ minutes, where circles and diamonds represent numerical results without surface binding and with receptors on the surface respectively.
Figure 5: The receptor-complexes due to ligand-receptor binding at 4 °C, (a) receptor-complex, and (b) receptor-complex dimer, at 5, 10, 20, 40, and 60 minutes.

Figure 6: The effect of HSPG on ligand-receptor binding at 4 °C, (a) the concentration of ligand after adding HSPG at 5, 10, 20, 40, and 60 minutes, and (b) the concentration of free receptor versus that of HSPG with concentrations of 0, 1.6 \times 10^4, 1.6 \times 10^5, 2.0 \times 10^5, 4.0 \times 10^5 respectively at 60 minutes.
Figure 7: Visualization of ligand transport in the capillary at the condition of 37°C and inlet velocity \( u = 10.4 \text{ mm/min} \ (u = 0.1732 \text{ mm/s}) \), (a) at \( t = 5 \) minutes, (b) at \( t = 10 \) minutes, (c) at \( t = 20 \) minutes, (d) at \( t = 40 \) minutes, and (e) at \( t = 60 \) minutes.
Figure 8: Visualization of ligand transport in the capillary at the condition of 37°C and inlet velocity $u = 5.2$ mm/min, (a) at $t = 5$ minutes, (b) at $t = 10$ minutes, (c) at $t = 20$ minutes, (d) at $t = 40$ minutes, and (e) at $t = 60$ minutes.
Figure 9: Visualization of ligand transport in the capillary at the condition of 37°C and inlet velocity $u = 2.08$ mm/min, (a) at $t = 5$ minutes, (b) at $t = 10$ minutes, (c) at $t = 20$ minutes, (d) at $t = 40$ minutes, and (e) at $t = 60$ minutes.