Published in final edited form as:

IEEE Trans Biomed Eng. 2009 September ; 56(9): 2147–2155. doi:10.1109/TBME.2008.2002109.

A Computational Model of FGF-2 Binding and HSPG Regulation Under Flow

Wensheng Shen,

Department of Computational Science, SUNY Brockport, Brockport, NY, 14420 USA e-mail: wshen@brockport.edu (see <http://www.cps.brockport.edu/~shen>).

Changjiang Zhang,

Department of Computer Science, University of Kentucky, Lexington, KY 40506 USA e-mail: czhanb@csr.uky.edu.

Michael Fannon,

Department of Ophthalmology and Visual Science, University of Kentucky, Lexington, KY 40506 USA e-mail: mw-fann2@email.uky.edu.

Kimberly Forsten-Williams, and

Department of Chemical Engineering, Virginia Polytechnic Institute & State University, Blacksburg, VA 24601, USA e-mail: kfw@vt.edu.

Jun Zhang

Department of Computer Science, University of Kentucky, Lexington, KY 40506 USA e-mail: jzhang@cs.uky.edu (see <http://www.cs.uky.edu/~jzhang>).

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 proteoglycans (HSPGs) under flow conditions within a cylindrical-shaped vessel or capillary. The model consists of a set of coupled nonlinear partial differential equations (PDEs) and a set of coupled nonlinear ordinary differential equations (ODEs). 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 growth factor transport and binding to receptors with/without the presence of heparan sulfate, as well as the effect of flow rate on growth factor-receptor binding. Our computational model may provide a useful means to investigate the impact of fluid flow on growth factor dynamics and, ultimately, signaling within the circulation.

Index Terms

Convection diffusion reaction; Fibroblast growth factor; Computer modeling; Heparan sulfate proteoglycan; Incompressible Flow

I. 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 [15]. FGF-2 and 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], [8], [15]. Under normal conditions, the levels of FGF-2 are found relatively low [8]. These levels can, however be substantially elevated in cases of disease and cancer [9], [22]. For example, high levels of FGF-2 have been found in the circulation when angiogenic tumors existed [17]. The temporal and spatial distribution of FGF-2 is elemental to normal development as well as disease control [8] 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, but convection was not involved. Previous models of FGF regulation by HSPG and heparin used a static planar geometry better approximating cell culture dishes rather than flow reactors or blood vessels [8], [11]–[13].

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 bioreactor which allows the long-term culture and study of endothelial cells under flow [20]. 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 [12], [13]. 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 (triads), which may further bind to generate FGF-2-FGFR-HSPG dimers. The previous work using this model is 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 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 behavior of competing growth factor-binding proteins on cell surface under flow conditions.

II. METHODS

A. 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.

1) Incompressible Navier-Stokes equations—Our model was designed to simulate fluid flow in a straight cylindrical tube (i.e., a model capillary or bioreactor). A reasonable assumption for typical fluids used in *vitro* bioreactor is that they are incompressible. Given our model design, it is convenient to write the governing equations in cylindrical coordinates. 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 radial momentum equation

$$\rho \frac{\partial u}{\partial t} + \rho u \frac{\partial u}{\partial r} + \rho v \frac{\partial u}{\partial x} = \rho g_r - \frac{\partial p}{\partial r} + \frac{1}{r} \frac{\partial}{\partial r} (r \tau_{rr}) + \frac{\partial \tau_{rx}}{\partial x}, \quad (1)$$

and the axial momentum equation

$$\rho \frac{\partial v}{\partial t} + \rho u \frac{\partial v}{\partial r} + \rho v \frac{\partial v}{\partial x} = \rho g_x - \frac{\partial p}{\partial x} + \frac{1}{r} \frac{\partial}{\partial r} (r \tau_{rx}) + \frac{\partial \tau_{xx}}{\partial x}. \quad (2)$$

In Eqs. (1) and (2), ρ is the density, u 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

τ_{rr} , τ_{rx} , and τ_{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 μ is a constant.

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, and the dissipation term describes diffusive transport of components due to the gradient of FGF-2 concentration. 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:

$$\frac{\partial \phi_i}{\partial t} + \frac{1}{r} \frac{\partial (r u \phi_i)}{\partial r} + \frac{\partial (v \phi_i)}{\partial x} = \frac{1}{r} \frac{\partial}{\partial r} \left(K_r r \frac{\partial \phi_i}{\partial r} \right) + \frac{\partial}{\partial x} \left(K_x \frac{\partial \phi_i}{\partial x} \right) + F_i(\phi_1 \dots \phi_n), \quad 1 \leq i \leq n, \quad (3)$$

where ϕ_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 ϕ_i is simply FGF-2. Molecular binding happens only on the tube surface, i.e. K_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 ($n=9$).

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 [9], [12], [13]. As was done previously [12], [13], we assumed that each cell has a homogeneous distribution of FGFR and HSPG binding sites [12]. 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 equations and Table I for reactions and parameters). The nine

nonlinear equations showing the concentration change of each species can be found in the Appendix.

B. Numerical Schemes

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 [10], [21] in the current computation. To solve the Navier-Stokes equations, two loops are required. The inner iteration handles each of the momentum equations, while the outer iteration deals with the coupling and nonlinearity. 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 [10]. In the transport equation, the convective term needs special treatments for stability consideration. A frequently used technique is upwind differencing. However, upwind discretization provides only first order spatial accuracy. A deferred correction numerical strategy was used here [10], 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 nonlinear ordinary differential equations were obtained from the set of biochemical reaction equations, in the form of mass action kinetics,

$\frac{d\phi}{dt} = \mathbf{f}(\phi, t)$. Newton's method is frequently used to solve nonlinear system, and the solution of the nonlinear system is

$$\phi^n = \phi^{n-1} + \Delta t (I - \Delta t J)^{-1} \phi^{n-1}, \quad (4)$$

where vector ϕ indicates the concentrations of species in the reaction network, J is the Jacobian

matrix, which is calculated as $J = \frac{\partial \mathbf{f}}{\partial \phi}$ 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. An analytical Jacobian is always preferred, since the computation of an 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 uses a variable-coefficient form of BDF (Backward Differencing Formulation) method. In the current computation, we used analytical Jacobian. The relative tolerance for stopping the VODE solver is set as 10^{-4} , and the absolute tolerance is species dependent, i.e., each species may have its own absolute tolerance.

III. RESULTS

A. Media flow in capillary and model validation

The FiberCell bioreactor is an excellent laboratory model for investigating endothelial cell function under flow. We therefore, chose to use it as a foundation for developing our computational model for investigating growth factor dynamics under flow although the model is not strictly dependent on the system specific parameters. The parameters of our model bioreactor are as follows, the radius of an individual fiber $R = 0.35\text{mm}$, the fiber length $L = 10\text{cm}$, the number of tubes or fibers per cartridge $N = 20$, the flow rate is adjustable in the range of $r = 80 \sim 0.5\text{ml/min}$. The viscosity is taken as $\mu = 0.04\text{ dyne.s/cm}^2$, about four times that of water, and the density of the fluid is taken as $\rho = 1060\text{ kg/m}^3$ [20]. We note that the effect of viscosity on the solution was minimal when values representative of water were used (data not

shown). The velocity range selected falls within the typical range used experimentally with the FiberCell bioreactor and that found *in vivo* for capillary blood flow [26].

The numerical solution for the flow was obtained by the 2D incompressible Navier-Stokes equations. Given the velocity, dimension and medium properties, the Reynold number is less than 1, and the flow within the capillary is laminar. The boundary conditions used for the flow simulation are prescribed as, a uniform flow velocity of $u = u_0$ at the tube entrance, non-slip boundary condition on the 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, which is a typical arrangement to extend the flow field outside the region of interest. We have verified that fluid can flow out of the capillary smoothly without affecting the velocity field inside the capillary (data not shown). 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 $u_{\max} = 2.0 \times u_{\text{inlet}}$. The steady-state flow field in two-dimension is presented in [25].

The accuracy of the numerical solution depends very much on the mesh size. 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 the 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. The radius of the fiber in our model is only 0.00035 m, more than 200 times smaller than the length. We found only a small (<0.05%) difference in FGF-2 concentration when 26 instead of 24 segments were used, indicating that 24 segments were sufficient in the radial direction. Figs. 3(a) and 3(b) are the concentration distribution of FGF-2 along the capillary surface at 10 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 paper, all numerical results are presented using a mesh size of 1400×24 .

B. Ligand transport and binding in the absence of HSPG

For our simulations, 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. 3, were as follows. At the tube entrance, a uniform concentration of $\phi = L_0 = 0.556$ nM was 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 $\frac{\partial \phi}{\partial x} = 0$, and such arrangement naturally extends concentration field outside the capillary. 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

$$\frac{\partial \phi}{\partial t} + \mathbf{n} \cdot \mathbf{F} = G \quad (5)$$

where ϕ 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 [11], the boundary condition for FGF-2 transport can be further expressed as

$$D \frac{\partial \phi}{\partial r} \Big|_{r=R} = (-k_f^R L R + k_r^R C + k_r^T T - L P - k_r^R G) / \text{CONST}, \quad (6)$$

where, D is the diffusivity taken as $9.2 \times 10^{-7} \text{ cm}^2/\text{s}$ estimated from the Stokes-Einstein equation [11], k_f^R , k_r^R , k_r^T , k_f^P , and k_r^R are parameters listed in Table I, and CONST is used for unit conversion, to convert the diffusion flux in unit of $\text{mol}/(\text{L}\cdot\text{s})$ to surface differential rate due to chemical reaction in unit of $\#/(\text{cell}\cdot\text{min})$. According to Elhadj et al. [7], bovine aortic endothelial cells could be cultured and seeded on the surface of a capillary in a cartridge of a bioreactor with a surface concentration of $2.9\text{b} \times 10^4 \text{ cells}/\text{cm}^2$. Based on this concentration, we can establish a unit conversion from (number of molecules)/cell to $\mu\text{mol}/\text{cm}^2$, i.e., 1 #/cell is equivalent to $4.82 \times 10^{-4} \mu\text{mol}/\text{cm}^2$.

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.* [13], 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 1400×24 , and it is grid independent. The computation results have been scaled with respect to the uniformly specified inlet FGF-2 boundary concentration, $L' = L/L_0$, where $L_0 = 0.0556 \text{ nM}$. 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 is transported by convection and diffusion down the capillary. The time-dependent solution of FGF-2 concentration at $t = 5, 10, 20$, and 40 minutes is shown in Figs. 4(a) ~ 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 [25]. As expected, without surface binding, the concentration of FGF-2 in the fluid phase at the capillary surface is the same as that in the solution, and reaches a constant at late times.

Next, we examine the impact of receptor binding on FGF-2 in solution. Assuming that receptors are attached on the capillary surface, FGF-2 molecules move from solution to capillary surface where they can bind with cell receptors. This is the initial value problem, which is modeled by assigning an initial condition of receptor concentration of $R_0 = 1.6 \times 10^4 \#/\text{cell}$ at the capillary surface. We present the concentration distribution of unbound FGF-2 in Figs. 4(a) ~ 4(d) when cellular receptors are present. Comparing the FGF-2 concentration at the capillary/cell surface

as a function of time when receptors are or are not present, one can clearly see that depletion is occurring on a relevant time scale.

Figs. 5(a) and 5(b) exhibit the dependency of FGF-2-FGFR and their dimers on location and time under 4°C conditions (no synthesis or internalization). Five time points are considered here, $t = 5, 10, 20, 40,$ and 60 minutes. For the cases of 5 and 10 minutes, FGF-2-FGFR complexes are seen only in a part of the capillary and a higher level of FGF-2-FGFR than FGF-2-FGFR dimer is evident. After 20 minutes, FGF-2-FGFR complexes can be seen down the length of the capillary as the velocity is high enough to permit exposure to FGF-2 throughout. At 60 min, higher densities of FGF-2-FGFR complexes are found as well as FGF-2-FGFR dimers. 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 shows a gradual development of FGF-2-FGFR dimers, suggesting signaling might be delayed beyond simple exposure time.

C. 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 $P_0 = 1.6 \times 10^6 \text{ \#}/\text{cell}$. The concentration of FGF-2 on capillary surface is displayed in Fig. 6(a) as a function of time. It is clear that the FGF-2 concentration is reduced significantly when HSPGs are present, by comparing the results in Fig. 4 and Fig. 6(a). The reduction in 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 of HSPG $0, 1.6 \times 10^4, 1.6 \times 10^5, 2.0 \times 10^5, 8.0 \times 10^5,$ and $1.6 \times 10^6 \text{ \#}/\text{cell}$ respectively. The results are plotted in Fig. 6 (b) in terms of the concentration of unbound receptors at $t = 60 \text{ min}$. Note that the results for the highest concentrations of HSPG were not shown since the resulting density of unbound receptors was less than 5 receptors/cell. The density of unbound receptor decreases with increasing HSPG, which is largely due to the binding between FGF-2-HSPG and FGFR to form FGF-2-FGFR-HSPG complexes and their dimers. The model shows that almost no free receptors can be found at the capillary surface when the HSPG concentration is increased to $4.0 \times 10 \text{ \#}/\text{cell}$ and above. This results from the constant supply of FGF-2 at the tube entrance enabling a high surface concentration to be maintained for cell interactions. As an outcome of this high FGF-2 concentration, free receptors are used up due to continuous binding between FGF-2 and receptors or binding between FGF-2-HSPG complexes and receptors. 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 coupling rate of FGF-2-FGFR and HSPG to form FGF-2-FGFR-HSPG complexes. The dependency of FGFR and its binding complexes on HSPG density is not linear. For example, the concentration change in FGFR due to an increase of HSPG from 0 to $1.6 \times 10^4 \text{ \#}/\text{cell}$ is not as significant as that of FGFR when the concentration of HSPG is increased from 1.6×10^4 to $1.6 \times 10^5 \text{ \#}/\text{cell}$, due to the importance of both binding and coupling towards effective ligand capture and receptor depletion. This observation may suggest that FGF-2 and receptor binding can be effectively regulated by carefully adjusting HSPG concentration.

D. 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. Fig.7, Fig.8 and Fig.9 are the time dependent concentration distribution of

ligand in the capillary of flow velocities 10.4 mm/min, 5.2 mm/min, and 2.08 mm/min respectively. All these three figures display the transient concentration field of FGF-2 in the capillary for the first hour as FGF-2 is added to the tube inlet at a constant rate. Five subfigures are included in each individual figure for different time intervals. Our results indicate that the FGF-2 distribution is greatly affected by flow velocity. An increased flow velocity results in higher 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. 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 about one eighth of the tube length in low velocity case (Fig. 9(a)). At $t = 60$ minutes, the volume-averaged normalized concentration of FGF-2 at tube exit is 0.67 in high velocity case, around 0.4 in middle velocity case, and 0.18 in the low velocity case.

IV. DISCUSSION AND CONCLUSION

Growth factors play a very important role in modulating cell activities of proliferation and differentiation. The MAP (mitogen-activated protein) kinase pathway starts with growth factor ligand binding to its transmembrane receptors followed by the activation of tyrosine kinases inherent in the receptor molecules [13], [24]. Such activation triggers the dimerization and phosphorylation of the transmembrane receptors. Computational modeling has been applied to many growth factor systems with the most developed being epidermal growth factor (EGF) systems [23], [27]. Less modeling work has been done for the heparin-binding growth factors such as FGF-2. Forsten *et al.* [12] and Fannon *et al.* [9] have previously studied the regulation of heparin and heparin-like molecules on FGF-2 binding in solution. Filion and Popel [11] proposed a model to address the effect of FGF-2 dimerization on surface interactions. In their one-dimensional diffusion-reaction model, 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. Their diffusion model does not take into account convection making it inadequate for flow situations found within the circulatory system or bioreactor such as the Cellmax system. Forsten-Williams *et al.* more recently proposed a kinetic model [13], 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. These efforts have focused on static culture systems, which differ from *in vivo* situations to some extent, where the concentrations of these molecules should be location dependent. This paper presents a dynamic model for FGF-2 binding on cell surface, where the FGF-2 concentrations are introduced under flow and not assumed to be uniform throughout the capillary. The motion of bio-fluids is fully considered. Currently we have adopted the FGF-2 surface binding model proposed by Forsten-Williams *et al.* [13], 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. Dimerization is viewed as a necessary first step in signal transduction for the majority of growth factors.

Our dynamic model puts FGF-2 binding in flow environment of a capillary, where FGF-2 molecules move with circulation flow inside capillary and bind to receptor and coreceptor molecules on tube surface. Under the current computation framework, the Reynolds number is far less than the critical value for transition to turbulence, and the capillary flow is typical Hagen-Poiseuille flow with the 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 coupled. Our numerical solution reveals that the concentrations of all species are indeed location dependent, as can be seen in Fig. 4, Fig. 5, Fig. 6, Fig. 7, Fig. 8, and Fig. 9 and reference [25]. 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 model and initial results may serve as a useful starting point.

In conclusion, a novel computational model was developed as a tool for investigating 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 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 thereafter 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 and binding. 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.

Acknowledgments

This research work was funded in part by NIH under grants R01-HL086644-01.

Appendix

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 [13]:

$$\frac{dR}{dt} = -k_f^R LR + k_r^R C + k_r^T T - k_c RG - k_{int} R + V_R, \quad (7)$$

$$\frac{dC}{dt} = k_f^R LR - k_r^R C - k_c CP - k_c C^2 + 2k_{uc} C_2 - k_{int} C, \quad (8)$$

$$\frac{dC_2}{dt} = \frac{k_c}{2} C^2 - k_{uc} C_2 - k_{int}^D C_2, \quad (9)$$

$$\frac{dT}{dt} = k_c RG + k_c CP - k_r^T T - k_c T^2 + 2k_{uc} T_2 - k_{int} T, \quad (10)$$

$$\frac{dT_2}{dt} = \frac{k_c}{2} T^2 - k_{uc} T_2 - k_{int}^D T_2, \quad (11)$$

$$\frac{dP}{dt} = -k_f^P LP + k_r^P G + k_r^T T - k_c CP - k_{int} P + V_p, \quad (12)$$

$$\frac{dG}{dt} = k_f^P LP - k_r^P G - k_c RG - k_c G^2 + 2k_{uc} G_2 - k_{int} G, \quad (13)$$

$$\frac{dG_2}{dt} = \frac{k_c}{2} G^2 - k_{uc} G_2 - k_{int} G_2, \quad (14)$$

$$V \frac{dL}{dt} = -k_f^R LR + k_r^R C + k_r^T T - k_f^P LP + k_r^P G \quad (15)$$

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, T_2 is FGF-2-HSPG-FGFR dimer, and V is defined as $V = 1.0 \times 10^{-9}$ L/cell.

REFERENCES

1. Browder T, Butterfield CE, Draling BM, Shi B, Marshall B, O'Reilly MS, Folkman J. Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. *Cancer Res* 2000;vol. 60:1878–1886. [PubMed: 10766175]
2. Brown PN, Byrne GD, Hindmarsh AC. VODE: A variable coefficient ODE solver. *SIAM J. on Sci. Comp* 1989;vol. 10:1038–1051.
3. Brown TD. Techniques for mechanical stimulation of cells in vitro: a review. *J. of Biome* 2000;vol. 33:3–14.
4. Chu CL, Goerges AL, Nugent MA. Identification of common and specific growth factor binding sites in heparan sulfate proteoglycan. *Biochemistry* 2005;vol. 44:12203–12213. [PubMed: 16142919]
5. Chua CC, Rahimi N, Forsten-Williams K, Nugent MA. Heparan sulfate proteoglycans function as receptors for fibroblast growth factor-2 activation of extracellular signal-regulated kinases 1 and 2. *Circu. Res* 2004;94:316–323.
6. Dowd CJ, Cooney CL, Nugent MA. Heparan sulfate mediates bFGF transport through basement membrane by diffusion with rapid reversible binding. *The J. of Bio. Chem* 1999;vol. 274:5236–5244.
7. Elhadj S, Akers RM, Forsten-Williams K. Chronic pulsatile shear stress alters insulin-like growth factor-I (IGF-I) binding protein release *in vitro*. *Ann. of Biom. Eng* 2003;vol. 31:163–170.
8. Fannon M, Forsten KE, Nugent MA. Potentiation and inhibition of bFGF binding by heparin: a model for regulation of cellular response. *Biochemistry* 2000;vol. 39:1434–1445. [PubMed: 10684625]
9. Fannon M, Forsten-Williams K, Dowd CJ, Freedman DA, Folkman J, Nugent MA. Binding inhibition of angiogenic factors by heparan sulfate proteoglycans in aqueous humor: potential mechanism for maintenance of an avascular environment. *The FASEB J* 2003;vol. 17:902–904.
10. Ferziger, JH.; Perić, M. *Comput. Meth. for Flu. Dyn.* Berlin, Germany: Springer; 1999.
11. Filion RJ, Popel AS. A reaction-diffusion model of basic fibroblast growth factor interactions with cell surface receptors. *Ann. of Biom. Eng* 2004;vol. 32:645–663.

12. Forsten KE, Fannon M, Nugent MA. Potential mechanisms for the regulation of growth factor binding by heparin. *J. of Theo. Biol* 2000;vol. 205:215–230.
13. Forsten-Williams K, Chua CC, Nugent MA. The kinetics of FGF-2 binding to heparan sulfate proteoglycans and MAP kinase signaling. *J. of Theo. Biol* 2005;vol. 233:483–499.
14. Gabhann FM, Yang MT, Popel AS. Monte Carlo simulations of VEGF binding to cell surface receptors in vitro. *Bioch. et Bioph. Acta* 2005;vol. 1746:95–107.
15. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996;vol. 86:353–364. [PubMed: 8756718]
16. Horowitz A, Tkachenko E, Simons M. Fibroblast growth factor-specific modulation of cellular response by syndecan-4. *J. of Cell Biol* 2002;vol. 157:715–725. [PubMed: 12011116]
17. Jerebtsova M, Wong E, Przygodzki R, Tang P, Ray PE. A novel role of Fibroblast Growth Factor-2 and Pentosan Polysulphate in the pathogenesis of intestinal bleeding in mice. *Amer. J. of Physi. - Heart and Circ. Physi* 2007;vol. 292:H743–H750.
18. Lovich MA, Edelman ER. Computational simulations of local vascular heparin deposition and distribution. *Amer. J. of Physi. - Heart and Circ. Physi* 1996;vol. 271:H2014–H2024.
19. Lovich MA, Philbrook M, Sawyer S, Weselcouch E, Edelman ER. Arterial heparin deposition: role of diffusion, convection, and extravascular space. *Amer. J. of Physi. - Heart and Circ. Physi* 1998;vol. 275:H2236–H2242.
20. Manuel, FiberCell Systems, Inc. <http://www.fibercellsystems.com>
21. Patankar, SV. Num. Heat Tran. and Flu. Flow. New York: The McGraw-Hill Company, Inc.; 1980.
22. Riley BB, Savage MP, Simandl BK, Olwin BB, Fallon JF. Retrobiral expression of FGF-2 (bFGF) affects patterning in chick limb bud. *Development* 1993;vol. 118:95–104. [PubMed: 8375342]
23. Schlessinger J. Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. *Cell* 2002;vol. 110:669–672. [PubMed: 12297041]
24. Schoeberl B, Eichler-Jonsson C, Gilles ED, Muller G. Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors. *Nat. Biot* 2002;vol. 20:370–375.
25. Shen, W. Ph.D Thesis. Lexington, KY: University of Kentucky; 2007. Computer Simulation and Modeling of Physical and Biological Processes Using Partial Differential Equations.
26. Tooke JE, Milligan DW. Capillary flow velocity in leukaemia. *Brit. Med. J* 1983;vol. 286:518–519.
27. Wiley HS, Shvartsman SY, Lauffenburger DA. Computational modeling of the EGF-receptor system: a paradigm for systems biology. *Tre. in Cell Biol* 2003;vol. 13:43–50.

(a)

(b)

Fig. 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, C=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.

Fig. 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 $x = 5.0\text{cm}$.

Fig. 3.

The dependency of numerical solution on mesh size in the case of surface reaction at 4 °C, $u_0 = 0.1732$ mm/s, and $L_0 = 0.0556$ nM, (a) at $t = 10$ minutes, and (b) at $t = 40$ minutes, where triangles, solid line, and circles represent numerical results of mesh size 1200×20 , 1400×24 , and $1600 \times$, 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.

Fig. 4. FGF-2 concentration on capillary surface in the cases of no surface binding and binding to its receptor only at 4 °C, $u_0 = 0.1732$ mm/s, and $L_0 = 0.556$ nM, (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.

Fig. 5. The receptor-bound complexes due to ligand-receptor binding at 4°C , $u = 0.1732$ mm/s, $L_0 = 0.556$ nM, and $R_0 = 1.6 \times 10^4$ #/cell, (a) FGF-2-FGFR complex, and (b) FGF-2-FGFR dimer, at 5, 10, 20, 40, and 60 minutes.

Fig. 6. The effect of HSPG on ligand-receptor binding at 4 °C, $u_0 = 0.1732$ mm/s, $L_0 = 0.0556$ nM, (a) the scaled concentration of ligand at the surface at various times when cells express both receptors and HSPG (1.6×10^6 #/cell, and (b) the concentration of free receptor with respect to various densities of HSPG, 0, 1.6×10^4 , 1.6×10^5 , 2.0×10^5 and 4.0×10^5 #/cell respectively at 60 minutes.

Fig. 7.

The concentration distribution of FGF-2 in the capillary at 37°C and $u_0 = 10.4$ mm/min (0.1732 mm/s) when cells express both receptors (1.6×10^4 #/cell) and HSPG (1.6×10^6 #/cell), where color scale indicates scaled concentration ($[L]/[L_0]$). The units of X and R are m, the concentration of FGF-2 is $L_0 = 0.556$ nM. The five subfigures are (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.

(a)

(b)

(c)

(d)

Fig. 8.

The concentration distribution of FGF-2 in the capillary at 37°C and $u_0 = 5.2$ mm/min when cells express both receptors (1.6×10^4 #/cell) and HSPG (1.6×10^6 #/cell), where color scale indicates scaled concentration ($[L]/[L_0]$). The units of X and R are m, the concentration of FGF-2 is $L_0 =$ nM. The five subfigures are (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.

(a)

(b)

(c)

(d)

Fig. 9.

The concentration distribution of FGF-2 in the capillary at 37°C and $u_0 = \text{mm/min}$ when cells express both receptors ($1.6 \times 10^4 \text{ \#}/\text{cell}$) and HSPG ($1.6 \times 10^6 \text{ \#}/\text{cell}$), where color scale indicates scaled concentration ($[L]/[L_0]$). The units of X and R are m, the concentration of FGF-2 is $L_0 = 0.556 \text{ nM}$. The five subfigures are (a) at $t = 5$ minutes, (b) at $t = 10$ minutes, (c) at $t = 20$ minutes, (d) at $t = 20$ minutes, and (e) at $t = 40$ minutes.

Table I

Chemical reactions included in the base model using mass-action kinetics and their relevant parameters [13]. The concentration of species on the tube surface can be converted from 1#/cell to $4.82 \times 10^{-14} \mu\text{mol}/\text{cm}^2$.

Chemical reaction	Kinetic formula	Parameters
$\begin{array}{c} k_f^R \\ L + R \rightleftharpoons C \\ k_r^R \end{array}$	$k_f^R[R][L], k_r^R[C]$	$k_f^R = 2.5 \times 10^8 \text{M}^{-1} \text{min}^{-1}, k_r^R = 0.048 \text{min}^{-1}$
$\begin{array}{c} k_f^P \\ L + P \rightleftharpoons G \\ k_r^P \end{array}$	$k_f^P[P][L], k_r^P[C]$	$k_f^P = 0.9 \times 10^8 \text{M}^{-1} \text{min}^{-1}, k_r^P = 0.068 \text{min}^{-1}$
$\begin{array}{c} k_c \\ R + G \rightarrow T \end{array}$	$k_c[R][G]$	$k_c = 0.001 \text{min}^{-1} (\#/\text{cell})^{-1}$
$\begin{array}{c} k_c \\ C + P \rightarrow T \end{array}$	$k_c[C][P]$	$k_c = 0.001 \text{min}^{-1} (\#/\text{cell})^{-1}$
$\begin{array}{c} k_r^T \\ T \rightarrow L + R + P \end{array}$	$k_r^T[T]$	$k_r^T = 0.001 \text{min}^{-1}$
$\begin{array}{c} k_c \\ C + C \rightleftharpoons C_2 \\ k_{uc} \end{array}$	$k_c[C]^2, k_{uc}[C_2]$	$k_c = 0.001 \text{min}^{-1} (\#/\text{cell})^{-1}, k_{uc} = 1 \text{min}^{-1}$
$\begin{array}{c} k_c \\ G + G \rightleftharpoons G_2 \\ k_{uc} \end{array}$	$k_c[G]^2, k_{uc}[G_2]$	$k_c = 0.001 \text{min}^{-1} (\#/\text{cell})^{-1}, k_{uc} = 1 \text{min}^{-1}$
$\begin{array}{c} k_c \\ T + T \rightleftharpoons T_2 \\ k_{uc} \end{array}$	$k_c[T]^2, k_{uc}[T_2]$	$k_c = 0.001 \text{min}^{-1} (\#/\text{cell})^{-1}, k_{uc} = 1 \text{min}^{-1}$
$\begin{array}{c} K_{int} \quad k_{int} \\ R \rightarrow R_{int}; P \rightarrow P_{int} \end{array}$	$k_{int}[R]; k_{int}[P]$	$k_{int} = 0.005 \text{min}^{-1}$
$\begin{array}{c} K_{int} \quad k_{int} \\ C \rightarrow C_{int}; G \rightarrow G_{int} \end{array}$	$k_{int}[C]; k_{int}[G]$	$k_{int} = 0.005 \text{min}^{-1}$
$\begin{array}{c} k_{int} \quad k_{int}^D \\ T \rightarrow T_{int}; C_2 \rightarrow C_{2int} \end{array}$	$k_{int}[T]; k_{int}^D[C_2]$	$k_{int} = 0.005 \text{min}^{-1}; k_{int}^D = 0.078 \text{min}^{-1}$
$\begin{array}{c} k_{int}^D \quad k_{int}^D \\ G_2 \rightarrow G_{2int}; T_2 \rightarrow T_{2int} \end{array}$	$k_{int}^D[G_2]; k_{int}^D[T_2]$	$k_{int}^D = 0.078 \text{min}^{-1}$