Intracoronary administration of FGF-2: a computational model of myocardial deposition and retention

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Filion, Renee J. and Aleksander S. Popel. Intracoronary administration of FGF-2: a computational model of myocardial deposition and retention. Am J Physiol Heart Circ Physiol 288: H263–H279, 2005. First published August 26, 2004; doi:10.1152/ajpheart.00205.2004.—This study uses a computational model to characterize the myocardial deposition and retention of basic fibroblast growth factor (FGF-2) at the cellular level after intracoronary (IC) administration of exogenous FGF-2. The model is applied to the in situ conditions present within the myocardium of a dog for which the plasma pharmacokinetics resulting from IC injection of FGF-2 were recorded. Our estimates show that the processes involved in FGF-2 signaling are not diffusion limited; rather, the response time is determined by the reaction time of FGF-2 binding to cell surface receptors. Additionally, the processes of receptor secretion and internalization are found to play crucial roles in the FGF-2 dynamics; future experiments are required to quantify these processes. The model predictions obtained in this study suggest that IC administration of FGF-2 via either a single bolus or repetitive injections causes a transient increase (time scale of hours) in myocardial FGF-2 concentration if the endogenous level of free interstitial FGF-2 is low enough to allow permeation of FGF-2 molecules from the microvascular to the interstitial spaces. The model shows that the majority (64%) of the extracellular FGF-2 ligands are located within the interstitium, and similar fractions are found in the basement membrane and extracellular matrix. Among the FGF-2 molecules found within the interstitium, 2% are free and 98% are bound to interstitial heparan sulfate proteoglycans. These results support the theory of extracellular control of the bioavailability of FGF-2 via dynamic storage of FGF-2 within the basement membrane and extracellular matrix.

basic fibroblast growth factor; mathematical; compartmental; angiogenesis; drug delivery

CARVIOCARDIOVASCULAR DISEASE, which ultimately leads to ischemic heart disease, is the number one cause of morbidity and mortality in the Western world (17, 46, 48). Present treatment options for patients suffering from ischemic heart disease include pharmacological interventions to reduce myocardial oxygen demand and revascularization procedures (coronary angioplasty or bypass surgery) to restore blood flow to a localized segment of the arterial tree (46, 48). However, a significant number of patients cannot be adequately managed by either pharmacological interventions or presently available revascularization procedures (45).

Recent advances in vascular biology have led to the development of therapeutic angiogenesis, which is a novel treatment strategy that relies on the stimulation of new blood vessel growth from preexisting vessels. Angiogenesis is a complex process that encompasses chemoattractant and mitogen activation, basement membrane breakdown, endothelial cell migration and proliferation, tube formation, and the deposition of a new basement membrane (81). A number of proangiogenic growth factors including fibroblast growth factors (FGFs) and vascular endothelial growth factors (VEGFs) have been identified as players in the angiogenic response to ischemia. In particular, basic FGF (FGF-2) was found to be an attractive candidate for therapeutic angiogenesis (45).

FGF-2 is a potent proangiogenic growth factor. It is highly conserved between species (40) and belongs to a large family of at least 23 related polypeptides (molecular mass, 17–34 kDa) that affect the growth, differentiation, migration, and survival of a wide variety of cells (13, 64, 67). FGF-2 is expressed at low levels in most organs and tissues in the body with higher concentrations in the brain and pituitary gland (56). Normal physiological FGF-2 concentrations are in the range of ≤1 ng/ml (20), and cell expression of FGF-2 can be up- or downregulated by the body.

FGF-2 is present in the heart at all developmental stages in the extracellular as well as the intracellular spaces (40). Specifically, both cardiac myocytes and endothelial cells in ventricular muscle contain FGF-2 (23). Lacking an amino-terminal signal peptide, FGF-2 is released from the cells as a monomer via an exocytotic mechanism that is independent of the endoplasmic reticulum/Golgi pathway (13, 67).

Two classes of FGF-2 receptors have been identified. The first class is composed of members of a family of tyrosine kinase receptors (FGFRs) found on cell surfaces. These receptors bind FGF-2 as well as other FGFs with high affinity but low capacity (63). Although the FGFR family is composed of four members (FGFR-1, -2, -3, and -4), only FGFR-1 is found in the heart (40, 57, 75). The second class of receptors is composed of heparan sulfate proteoglycans (HSPGs), which are found on cell surfaces as well as in extracellular matrix and basement membranes. These receptors bind FGF-2 as well as other FGFs with low affinity but high capacity (36, 73). Among the members of this family, syndecan-3 and glypican are abundantly expressed in the heart with lower levels of syndecan-2 and very low levels of syndecan-1 (2).

FGF-2-induced angiogenesis has been studied in many animal models including swine (46), dog (50, 51), and rat (16). The general results from these animal studies were promising. Human FGF-2 clinical trials were subsequently performed for myocardial ischemia, intermittent claudication, and peripheral vascular disease (45, 52, 62). Interestingly, although promising results were obtained in a few relatively small human clinical
trials, the larger, well-controlled human clinical trials did not show promising results. In fact, the largest clinical trial of FGF-2 administration in humans, the FGF Initiating Revascularization Trial (known as FIRST), concluded that administration of a single intracoronary (IC) infusion of FGF-2 in humans suffering from ischemic heart disease did not improve myocardial perfusion (77, 78). Based on this negative result along with similar negative results obtained from other large, well-controlled studies whereby growth factors such as VEGF were administered in humans, a reevaluation of the present approaches to therapeutic angiogenesis was recommended (77, 79). The topics to be reconsidered and/or studied in greater depth included the underlying biological principles, the choice of angiogenic agents, the validity and interpretation of animal model data, the translation of animal models to clinical trials, the optimal dose schedule (single vs. repeated or sustained delivery), and the mode of growth-factor delivery.

Therapeutic angiogenesis remains a promising medical concept. However, it is now clear that the complex process of angiogenesis must be understood in greater detail before clinical trials are initiated. Because experiments are limited in their ability to measure multiple parameters concurrently, particularly at the cellular level, a modeling approach is required.

The first model of FGF-2 binding kinetics was presented in 2000 (26). This model used a mass-balance approach to investigate the heparin regulation of FGF-2 binding to FGFRs on the cell surface of cultured fibroblasts at 4°C. The binding of FGF-2 to high-affinity FGFRs and low-affinity HSPGs was considered along with the formation of FGF-2/HSPG/FGFR triads (FGF-2 bound to an FGFR and stabilized by a nearby HSPG). The production of cell surface receptors and the internalization of cell surface species were assumed negligible at 4°C.

This FGF-2 model was recently extended to study the binding inhibition of FGF-2 by HSPGs in the aqueous humor (19). This extended model considered the production of cell surface receptors and the internalization of cell surface species. However, it did not account for the effects of temperature on the experimentally determined kinetic rate constants (measured at 4°C).

Based on these FGF-2 models and the available experimental data, we developed a comprehensive model of the FGF-2 binding kinetics for cultured fibroblast cells (22). This continuum reaction-diffusion model simulated the in vitro setting in which a confluent monolayer of Balb/c3T3 cells was grown in a single well of a 24-well plate and was in contact with a fluid layer that contained FGF-2 ligands and soluble receptors. Unique features of this model included the degradation of internalized cell surface species, the formation of 2 FGF-2/HSPG/FGFR double triads (two FGF-2 ligands bound to an FGFR and stabilized by a nearby HSPG), and the dimerization of FGF-2 ligands. Moreover, all experimentally determined kinetic rate constants and diffusivity values were scaled to 37°C. Results from this in vitro study suggested that the FGF-2-induced cellular response is the result of a temporal combination of cell surface triads, double triads, and FGF-2-bound HSPGs. Although ligand dimerization was shown to potentially regulate FGF-2 activity by shifting the distribution of signaling complexes from the less-stable triads to the more-stable double triads, the available experimental values for the self-association rate of FGF-2 monomers suggested negligible dimer formation.

In this study, we describe the first in vivo model developed to characterize the myocardial deposition and retention of FGF-2 at the cellular level after IC administration of FGF-2. Our model is applied to the in situ conditions present within the myocardium of a dog for which the pharmacokinetics resulting from IC injection of FGF-2 were recorded (51). The model utilizes the experimental data to make specific predictions that could motivate additional experiments and assist in the design and assessment of angiogenesis-based pharmacological interventions.

**Glossary**

| [species] | Concentration of species (in pmol/cm³ tissue) |
| q_F | FGF-2 cell surface secretion rate (in pmol·cm⁻³ tissue·min⁻¹) |
| p_F | FGF-2 permeation rate across capillary wall (in pmol·cm⁻³ tissue·min⁻¹) |
| s_0 | FGFR production rate (in pmol·cm⁻³ tissue·min⁻¹) |
| s_H | HSPG production rate (in pmol·cm⁻³ tissue·min⁻¹) |
| k_on | Association rate constant [in (pmol/cm³ tissue)]⁻¹·min⁻¹ |
| k_off | Dissociation rate constant (in min⁻¹) |
| k_e | Internalization rate constant for cell surface species (in min⁻¹) |
| k_0 | Degradation rate constant for internalized complexes (in min⁻¹) |

**MODEL DEVELOPMENT**

**Geometry**

As a first approximation, the myocardium can be represented as a collection of capillaries (or small venules), each of which is surrounded by several parenchymal cells (6, 41). A schematic of a typical capillary with neighboring parenchymal cells is presented in Fig. 1A. For simplicity, the capillary endothelium is represented as a concentric cylinder surrounded by another concentric cylinder that represents the interstitial space between the endothelial and parenchymal cells. The parenchymal cells, which are assumed to be myocytes, are further separated by thin interstitial spaces.

Figure 1B illustrates the different components of the interstitial space. The myocardial capillary is a thin-walled tube of endothelial cells one layer thick that rests on a basement membrane (EBM). A similar basement membrane is associated with the surface of the myocytes (MBM), and the two basement membranes are separated by an extracellular matrix (ECM). Together, the EBM, ECM, and MBM make up the interstitial space that binds the cells in the myocardial tissue and serves as a reservoir for many hormones and growth factors.

Figure 1C illustrates the species found in the interstitial space as well as on the endothelial and myocyte cell surfaces. A uniform distribution of HSPGs is assumed within each of the basement membranes and the ECM, with a higher concentration in the basement membranes (3, 14). Uniform distributions of low-affinity (HSPG) and high-affinity (FGFR) receptors are also assumed on both the endothelial and myocyte cell surfaces.
The flat endothelial cells that constitute the myocardial capillary are separated by narrow spaces called intercellular clefts (82). These intercellular clefts allow the exchange of molecules such as FGF-2 between the capillary lumen and the interstitial space (49).

At the beginning of the simulation, all interstitial and cell surface species were set to their basal concentrations (steady-state concentrations obtained without administration of exogenous FGF-2). A single bolus injection of exogenous FGF-2 was administered into the coronary circulation at time \( t = 0 \) h. Subsequent to this injection, the myocardial capillary plasma concentration of FGF-2 decreased as a function of time according to the experimental recordings reported by Lazarous et al. (51).

**Reaction Kinetics**

Figure 2 summarizes the reaction pathways included in our model. The reaction scheme presented in Fig. 2 is similar to the scheme used in our previous in vitro model and is based on the same large body of experimental literature (see Ref. 22).

Within the interstitium, the FGF-2 ligands \( F \) can interact with the interstitial HSPGs \( h \) to form FGF-2/interstitial HSPG complexes \( Fh \). At the endothelial or myocyte cell surfaces, the FGF-2 ligands can interact with unoccupied cell surface species to form various complexes. An \( F \) can bind to either an unoccupied FGFR \( R \) or an unoccupied HSPG \( H \) to form an FGF-2/FGFR \( FR \) or FGF-2/HSPG \( FH \) complex, respectively. The \( FR \) and \( FH \) complexes can then diffuse along the plasma membrane and couple to a nearby unoccupied \( H \) and \( R \), respectively, to form FGF-2/HSPG/FGFR triads \( FHR \). The binding of a second FGF-2 ligand after triad formation results in the formation of the more stable 2 FGF-2/HSPG/FGFR double triad \( FFHR \).

Once formed, all cell surface complexes are either dissociated back into interstitial and cell surface species or they are internalized. Although not depicted in Fig. 2, both the endothelial cells and the myocytes are constantly producing FGFRs and HSPGs. This production counters the internalization of receptors. Moreover, both endothelial cells and myocytes are constantly secreting FGF-2 ligands that are free to bind to cell surface receptors or nearby interstitial HSPGs. This secretion counters the degradation of internalized FGF-2 complexes.

**Compartmental Model**

FGF-2 is a small, globular protein with a molecular radius of 1.45 nm (14). We show (see Appendix C, Model Justification) that the diffusion gradients of free ligands are small and the diffusion gradients of free ligands are small and the
diffusion times are short (compared with association and dissociation times). Based on these estimates, the spatial gradients of free FGF-2 ligands are neglected (diffusion is assumed instantaneous), and our model is simplified to the compartmental model presented in Fig. 3.

All species concentrations are reported in units of picomoles per cubic centimeter of myocardial tissue. Divided into nine compartments, the model considers the free FGF-2 concentration within the microvascular space (subscript L), the free FGF-2 concentration within the interstitial space (no subscript), the endothelial and myocyte cell surface species concentrations (subscripts E and M, respectively), the endothelial and myocyte internalized species concentrations (subscripts intE and intM, respectively), and the concentrations of bound and unbound interstitial HSPGs within the EBM, ECM, and MBM (subscripted accordingly).

Fig. 3. Compartmental model of the myocardium. This model is composed of nine compartments that represent the free FGF-2 concentration within the microvascular space (subscript L), the free FGF-2 concentration within the interstitial space (no subscript), the endothelial and myocyte cell surface species concentrations (subscripts E and M, respectively), the endothelial and myocyte internalized species concentrations (subscripts intE and intM, respectively), and the concentrations of bound and unbound interstitial HSPGs within the EBM, ECM, and MBM (subscripted accordingly).

Equations

The binding kinetics depicted in Fig. 2 are represented by a set of ordinary differential equations. These equations, which describe the changes in species concentrations as a function of time, are similar to those used in our previous in vitro FGF-2 model (22) but are extended to the in vivo geometry.

The changes in endothelial cell surface species concentrations with respect to time are given by

\[
\frac{\partial [R_E]}{\partial t} = s_{R,E} - k_{o_{R,E}}[F][R_E] - k_{o_{FR,E}}[FR_E][R_E] + k_{o_{FR,E}}[FR_E] + k_{o_{FFHR,E}}[FFHR_E] - k_{R,E}[R_E] \tag{1}
\]

\[
\frac{\partial [H_E]}{\partial t} = s_{H,E} - k_{o_{H,E}}[F][H_E] - k_{o_{FR,E}}[FR_E][H_E] + k_{o_{FR,E}}[FR_E] + k_{o_{FFHR,E}}[FFHR_E] - k_{H,E}[H_E] \tag{2}
\]

\[
\frac{\partial [FR_E]}{\partial t} = k_{o_{FR,E}}[FR_E] - k_{o_{FR,E}}[FR_E][H_E] - k_{o_{FR,E}}[FR_E] - k_{o_{FR,E}}[FR_E] \tag{3}
\]
The changes in internalized species concentrations with respect to time are given by

\[ \frac{\partial [FHR_{\text{int}}]}{\partial t} = k_{\text{onFH,R}} [FHR_{\text{M}}] - k_{\text{degFH,M}} [FHR_{\text{int}}] \]  

(6)

which represent the production of FGFRs and HSPGs along with the association, dissociation, and internalization of all cell surface species. The internalized cell surface complexes are either degraded by lysosomes or translocated to the nucleus. Therefore, the changes in internalized species concentrations with respect to time are given by

\[ \frac{\partial [FHR_{\text{int}}]}{\partial t} = k_{\text{onFH,R}} [FHR_{\text{M}}] - k_{\text{degFH,M}} [FHR_{\text{int}}] \]  

(7)

\[ \frac{\partial [FH_{\text{int}}]}{\partial t} = k_{\text{onFH,E}} [FH_{\text{M}}] - k_{\text{degFH,M}} [FH_{\text{int}}] \]  

(8)

\[ \frac{\partial [FR_{\text{int}}]}{\partial t} = k_{\text{onFR,M}} [FR_{\text{M}}] - k_{\text{degFR,M}} [FR_{\text{int}}] \]  

(9)

\[ \frac{\partial [FFHR_{\text{int}}]}{\partial t} = k_{\text{onFH,M}} [FFHR_{\text{M}}] - k_{\text{degFH,M}} [FFHR_{\text{int}}] \]  

(10)

Similarly, the changes in myocyte cell surface species concentrations with respect to time are given by

\[ \frac{\partial [R_{\text{M}}]}{\partial t} = s_{\text{FM}} - k_{\text{onFR,M}} [F][R_{\text{M}}] - k_{\text{onFH,M}} [FH_{\text{M}}][R_{\text{M}}] \]  

\[ + k_{\text{offFH,M}} [FR_{\text{M}}] + k_{\text{offFR,M}} [FHR_{\text{M}}] - k_{\text{RM}} [R_{\text{M}}] \]  

(11)

\[ \frac{\partial [H_{\text{M}}]}{\partial t} = s_{\text{HM}} - k_{\text{onFH,M}} [F][H_{\text{M}}] - k_{\text{onFR,M}} [FR_{\text{M}}][H_{\text{M}}] \]  

\[ + k_{\text{offFH,M}} [FH_{\text{M}}] + k_{\text{offFR,M}} [FHR_{\text{M}}] - k_{\text{HM}} [H_{\text{M}}] \]  

(12)

\[ \frac{\partial [FR_{\text{M}}]}{\partial t} = k_{\text{onFR,M}} [F][R_{\text{M}}] - k_{\text{onFH,E}} [FH_{\text{M}}][R_{\text{M}}] \]  

\[ - k_{\text{offFH,M}} [FR_{\text{M}}] - k_{\text{offFR,M}} [FR_{\text{M}}] \]  

(13)

\[ \frac{\partial [FH_{\text{M}}]}{\partial t} = k_{\text{onFH,E}} [F][H_{\text{M}}] - k_{\text{onFH,M}} [FH_{\text{M}}][R_{\text{M}}] \]  

\[ - k_{\text{offFH,M}} [FH_{\text{M}}] - k_{\text{offFH,M}}[FH_{\text{M}}][H_{\text{M}}] \]  

(14)

\[ \frac{\partial [F]}{\partial t} = p_{F} + q_{F,E} - k_{\text{offFH,M}} [F][H_{\text{M}}] + k_{\text{offFH,E}} [F][H_{\text{M}}] + k_{\text{offFH,M}}[F][H_{\text{M}}] + k_{\text{offFH,E}}[F][H_{\text{M}}] - k_{\text{offFH,M}}[F][R_{\text{M}}] \]  

\[ - k_{\text{offFH,M}}[F][H_{\text{M}}] - k_{\text{offFH,M}}[F][H_{\text{M}}][R_{\text{M}}] \]  

(27)

reflecting the binding and unbinding of FGF-2 to interstitial HSPGs within the EBM, ECM, and MBM, respectively. The total number of interstitial HSPGs is assumed to be conserved (no degradation of interstitial HSPGs). Thus the concentrations of FGF-2-bound HSPGs are subtracted from the total number of interstitial HSPGs to obtain

\[ h_{\text{EBM}} = h_{\text{EBM}} - [FH_{\text{EBM}}] \]  

(24)

\[ h_{\text{ECM}} = h_{\text{ECM}} - [FH_{\text{ECM}}] \]  

(25)

\[ h_{\text{MBM}} = h_{\text{MBM}} - [FH_{\text{MBM}}] \]  

(26)

which represents the concentrations of free interstitial HSPGs within the EBM, ECM, and MBM, respectively.

Finally, the change in the interstitial concentration of free FGF-2 with respect to time is given by
which reflects the permeation of FGF-2 from the microvascular to the interstitial spaces, the secretion of FGF-2 from the cell surfaces, and the binding and unbinding of FGF-2 to and from interstitial and cell surface receptors.

**Parameters**

An extensive literature search was conducted to compile a list of experimentally determined model parameter values (or, if unavailable, representative values) reflecting the in vivo conditions within the myocardium. The parameter values used in our model are listed online (at http://ajpheart.physiology.org/cgi/content/full/00205.2004/DC1) and are discussed in detail in Appendix A. This is the first such compilation of these values. Note that an effort has been made to use canine experimental values whenever possible. When such values were unavailable, a cross section of experimental values obtained for a variety of different species was collected, and a representative average value was selected.

To investigate the changes that occur within the myocardium after IC injection of exogenous FGF-2, the basal state of the myocardium must be determined before FGF-2 administration. Thus a preliminary basal state simulation was performed to determine the steady-state (no exogenous FGF-2) concentrations of all species included in the model.

Owing to the poor extractability of FGF-2 from tissue, there is uncertainty regarding the absolute concentration of endogenous FGF-2 present in heart (9, 10). FGF-2 concentrations ranging from 6 to 13 μg/kg of tissue were originally reported for the healthy adult bovine heart (70). However, a more recent study reported an average FGF-2 concentration of 228.5 μg/kg of tissue (10), which suggests that the absolute concentration of endogenous FGF-2 present in the heart is considerably higher than previously estimated. As a result of this uncertainty, model predictions are presented for both low and high basal levels of FGF-2 (F_o,basal) within the myocardial tissue (6 and 228.5 μg/kg of tissue, respectively).

The basal state simulation was run for a total duration of 60 h (with low F_o,basal) and 100 h (with high F_o,basal), whereupon the concentrations of all species included in the model were assumed to have reached steady state. These assumed steady states were verified by repeating the basal state simulations but with all FGF-2 ligands initially bound to interstitial HSPGs. Fractional differences (no bound ligands vs. no free ligands) of <2.2 × 10^{-6} (for low F_o,basal) and <2.7 × 10^{-4} (for high F_o,basal) were obtained for all species concentrations. Additionally, fractional differences of <7.0 × 10^{-4} (for low F_o,basal) and <3.0 × 10^{-2} (for high F_o,basal) were obtained for all species concentrations reported within ±10 h of the assumed steady states.

The steady-state concentrations obtained from the low and high F_o,basal basal state simulations are listed in Table 1. These basal concentrations were used as the initial conditions in the corresponding IC administration simulations. In these simulations, a single-bolus injection of exogenous FGF-2 was administered into the coronary circulation at t = 0 h. The time-varying concentration of FGF-2 within the microvascular space was set to

\[ [F]_t = (4.85 \times 10^{-4}) \cdot t^{-0.9978} \]

where \([F]_t\) is the luminal concentration of FGF-2 (in μM), and \(t\) is the time elapsed since the IC injection (in min). Equation 28 corresponds to a trend line fit to the experimental data reported by Lazarous et al. (51) for a single bolus injection of FGF-2 into the coronary circulation of a mongrel dog. The experimental data and trend line are illustrated in Fig. 4. All other parameter values were set to their default values (which can be found in the online data supplement at http://ajpheart.physiology.org/cgi/content/full/00205.2004/DC1).

**Table 1. Steady-state concentrations of all species included in model**

<table>
<thead>
<tr>
<th>Species</th>
<th>Low Initial FGF-2</th>
<th>High Initial FGF-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intersitial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(F)</td>
<td>1.93×10^{-3}</td>
<td>0.16</td>
</tr>
<tr>
<td>(F_{BBM})</td>
<td>1.18×10^{-2}</td>
<td>0.94</td>
</tr>
<tr>
<td>(\bar{F}_{BM})</td>
<td>33.08</td>
<td>32.15</td>
</tr>
<tr>
<td>(F_{CBM})</td>
<td>5.88×10^{-2}</td>
<td>4.69</td>
</tr>
<tr>
<td>(\bar{F}_{CM})</td>
<td>164.21</td>
<td>159.58</td>
</tr>
<tr>
<td>(F_{BBBM})</td>
<td>2.52×10^{-2}</td>
<td>2.01</td>
</tr>
<tr>
<td>(\bar{b}_{BBM})</td>
<td>70.50</td>
<td>68.52</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R_{E})</td>
<td>0.14</td>
<td>3.44×10^{-3}</td>
</tr>
<tr>
<td>(R_{H})</td>
<td>10.92</td>
<td>10.17</td>
</tr>
<tr>
<td>(F_{R})</td>
<td>1.70×10^{-4}</td>
<td>3.53×10^{-4}</td>
</tr>
<tr>
<td>(F_{HR})</td>
<td>7.96×10^{-3}</td>
<td>0.62</td>
</tr>
<tr>
<td>(FFHR_{E})</td>
<td>6.98×10^{-3}</td>
<td>3.00×10^{-3}</td>
</tr>
<tr>
<td>(FFHR_{H})</td>
<td>5.81×10^{-4}</td>
<td>2.05×10^{-2}</td>
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<tr>
<td>(FR_{m})</td>
<td>6.65×10^{-4}</td>
<td>1.38×10^{-4}</td>
</tr>
<tr>
<td>(FH_{m})</td>
<td>7.11×10^{-3}</td>
<td>0.55</td>
</tr>
<tr>
<td>(FHR_{m})</td>
<td>5.36×10^{-3}</td>
<td>2.30×10^{-2}</td>
</tr>
<tr>
<td>(FFHR_{m})</td>
<td>4.46×10^{-3}</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Myocytes                       |                  |                   |
| \(R_{M}\)                     | 0.21             | 4.43×10^{-3}      |
| \(H_{M}\)                     | 32.30            | 30.28             |
| \(F_{R}\)                     | 1.94×10^{-4}     | 3.56×10^{-4}      |
| \(F_{H}\)                     | 2.37×10^{-2}     | 1.85              |
| \(FHR_{M}\)                   | 1.22×10^{-3}     | 4.67×10^{-3}      |
| \(FFHR_{M}\)                  | 1.01×10^{-3}     | 3.19×10^{-2}      |
| \(FR_{m}\)                    | 7.55×10^{-4}     | 1.39×10^{-3}      |
| \(FH_{m}\)                    | 2.12×10^{-2}     | 1.65              |
| \(FHR_{m}\)                   | 9.35×10^{-2}     | 3.58×10^{-2}      |
| \(FFHR_{m}\)                  | 7.78×10^{-3}     | 0.24              |

Results are from basal state simulations. Low and high basal fibroblast growth factor (FGF-2) concentrations were 0.35 and 13.46 pmol/cm^3 tissue, respectively. \(F\), FGF-2; \(F_{H}\), FGF-2/interstitial heparan sulfate proteoglycans (HSPG) complex; \(h\), interstitial HSPG; \(R\), FGF-2 receptor; \(H\), HSPG; \(FR\), FGF-2/FGFR complex; \(FH\), FGF-2/HSPG complex; \(FFHR\), FGF-2/HSPG/FGF co-receptor; \(FHR\), 2 FGF-2/HSPG/FGFR double-trioid complex; \(R\), endothelial basement membrane; \(E\), extracellular matrix; \(E\), endothelial cell surface; \(M\), myocyte; \(intE\) and \(intM\), internalized endothelial and myocyte concentrations, respectively.

**Implementation**

The model developed in this study was implemented using Matlab 6.0 software (Mathworks; Natick, MA). Transient solutions to the set of ordinary differential equations (see Equations) were obtained using Matlab’s built-in stiff differential equation solver ODE15s. A relative error tolerance of 10^{-10} was used along with a uniform absolute error tolerance of 10^{-15}.

**RESULTS**

**Basal State of Myocardium**

A preliminary basal state simulation was performed to determine the steady-state (no exogenous FGF-2) concentrations...
of all species included in the model. Initially, all FGF-2 ligands were unbound (no preformed complexes within the interstitium or on the cell surfaces). The resulting basal concentrations of all species included in the model are listed in Table 1. The temporal results of the basal state simulation with low $F_{o,basal}$ value are presented in Figs. 5 and 6. Figure 5 depicts the changes in interstitial complex concentrations as a function of time, whereas Fig. 6, A and B (for endothelial cell) and C and D (for myocyte) depict the changes in cell surface and internalized complex concentrations, respectively, as a function of time.

At $t = 0 \text{ h}$, all FGF-2 ligands are uniformly distributed within the interstitium and unbound (in other words, there are no preformed complexes). The binding of FGF-2 to interstitial HSPGs occurs rapidly; within 6 s, 98% of the free FGF-2 ligands were bound to basement membrane or ECM HSPGs (forming $F_h$ complexes). This behavior is characterized by the spikes depicted in Fig. 5. Note that although the density of HSPGs in the EBMs and MBMs is 17 times greater than in the ECM, the ECM occupies significantly more interstitial space than the EBMs and MBMs combined (97% ECM vs. 3% BM). As a result, 62% of all interstitial HSPGs are located in the ECM, leaving 12% in the EBM and 26% in the MBM. Thus, as expected, 12, 62, and 26% of all interstitial HSPG-bound FGF-2 ligands are found within the EBM, ECM, and MBM, respectively.

The bound FGF-2 ligands eventually dissociate from the interstitial HSPGs and diffuse to the cell surfaces. Owing to the abundance of cell surface HSPGs, large $F_h$ concentrations form on the endothelial and myocyte cell surfaces (Fig. 6, A and C). Much smaller concentrations of $F_r$ complexes also form on the endothelial and myocyte cell surfaces. Similar to the results of our previous in vitro model, the $F_r$ and $F_h$ complexes are rapidly converted into $FHR$ triads. These $FHR$ triads are then converted into $FFHR$ double triads. The ongoing internalization of the cell surface species results in large concentrations of receptor-bound FGF-2 complexes within the endothelial and myocyte cells (Fig. 6, B and D). Note that the lysosomal degradation of internalized FGF-2 complexes is included in the model equations. Thus the internalized complex concentrations depicted in the model results correspond to the internalized complexes translocated to the nucleus. These nuclear complexes are thought to contribute at least in part to the biological activity of the growth factor (11, 80).

The results obtained from the basal state simulation with high $F_{o,basal}$ values are similar to those obtained from the basal state simulation with low $F_{o,basal}$ values and are therefore omitted for the sake of brevity.

Experimental observations have shown that FGF-2 is deposited in the basement membranes of vascular endothelial cells and cardiac myocytes as well as in the corresponding ECM (84). Combined with the high concentrations of HSPGs found in the basement membranes and ECM (14), these experimental observations lead to the hypothesis that the basement membranes and ECM serve as dynamic storage sites for FGF-2 (3, 14, 24, 75, 83, 84). The bioavailability of FGF-2 is thus controlled extracellularly by the rapid and reversible binding of FGF-2 to the interstitial HSPGs.

The predicted basal state of the myocardium reflects these experimental observations. Of all the FGF-2 molecules in the modeled myocardium with low $F_{o,basal}$ value, 28% are found within the interstitium, 57% are found internalized and translocated to the nuclei of the endothelial and myocyte cells, and 15% are bound to endothelial and myocyte cell surface receptors. Among the FGF-2 molecules outside the cells (i.e., not internalized), 36% are bound to cell surface receptors and 64% are found within the interstitium. A similar distribution was obtained in the modeled myocardium with high $F_{o,basal}$ value. In both simulations, 98% of the FGF-2 molecules found within the interstitium are bound to interstitial HSPGs. Therefore, because most of the FGF-2 molecules found outside the cells...
are within the interstitium and most of those are bound to interstitial HSPGs, few FGF-2 ligands are free to bind to cell surface receptors. This result agrees with the proposed extra-cellular control of the bioavailability of FGF-2.

Single Bolus IC FGF-2 Administration

The basal steady-state concentrations of all species included in the model, obtained via the basal state simulations with low and high \( F_{o,\text{basal}} \) values, were used as the initial concentrations in the corresponding IC administration simulations.

The temporal results of the IC administration simulation with low \( F_{o,\text{basal}} \) values are presented in Figs. 7 and 8. Figure 7A depicts the changes in free interstitial FGF-2 concentration and Fig. 7B shows the changes in interstitial complex concentrations as functions of time. Figure 8, A and B (for endothelial cell) and C and D (for myocyte) depict the changes in cell surface and internalized complex concentrations, respectively, as functions of time.

At \( t = 0 \) h, a single bolus injection of exogenous FGF-2 was administered into the coronary circulation. The exogenous FGF-2 molecules rapidly permeated through the capillary walls and entered the myocardial interstitial space. Within 23 min of the injection, the total number of FGF-2 molecules within the interstitium reached a maximum (see Fig. 7). At this point, there were approximately three times more FGF-2 molecules in the interstitial space than in the basal state. As in the basal state, 98% of all interstitial FGF-2 molecules were bound to matrix HSPGs.

Once in the interstitial space, the FGF-2 molecules can bind to the endothelial and myocyte cell surfaces. Owing to the abundance of cell surface HSPGs, large \( FH \) concentrations formed on the endothelial and myocyte cell surfaces (Fig. 8, A and C). Many of the \( FH \) complexes were rapidly converted to \( FHR \) triads, which led to large cell surface and internalized \( FHR \) concentrations.

As with intravenous injections, the IC delivery method results in significant systemic recirculation (47, 77). Owing to
this recirculation process, whereby the majority of the injected FGF-2 is deposited in the liver (51), the coronary plasma concentration of exogenous FGF-2 decreases rapidly. Ultimately, it is this recirculation process that leads to the low uptake and retention of exogenous FGF-2 in the myocardium. Our model predictions obtained from the IC administration simulation with low $F_{o,\text{basal}}$ value reflect these experimental observations.

As shown in Figs. 7 and 8, the increases in the interstitial and cell-associated FGF-2 concentrations drop off within the first 10 h. The myocardium then gradually returns to its basal state and reaches within 5% of its basal state 20 h after injection and attains 1% of its basal state 26 h after injection.

The IC administration simulation was also performed with high $F_{o,\text{basal}}$ value. In this case, the endogenous level of free FGF-2 present within the interstitium of the myocardium was quite high. Thus subsequent to the IC injection, very little additional (exogenous) FGF-2 permeated from the microvascular to the interstitial spaces. A maximum concentration of free interstitial FGF-2, which was only slightly greater than the original concentration, was achieved within 1 min of the injection. This minimal increase in free FGF-2 concentration results in minimal changes in FGF-2-bound complex concentrations. Flat curves were obtained in the temporal plots of endothelial and myocyte cell surfaces and internalized complex concentrations (not shown).

Repetitive IC Administration of FGF-2

Based on the results obtained from our IC administration simulations, the IC injection of a single bolus of exogenous FGF-2 may transiently increase the total concentration of FGF-2 within the myocardium (depending on the level of endogenous FGF-2 free within the interstitium before the injection); within hours, the concentration in the myocardium returns to its basal state. To investigate the effects of multiple repetitive injections on the state of the myocardium, a simulation of two successive injections of exogenous FGF-2 was performed. Note that this simulation was performed with low $F_{o,\text{basal}}$ value, because high $F_{o,\text{basal}}$ value produces negligible changes in the modeled species concentrations.

The IC administration simulation was performed (with a bolus injection of exogenous FGF-2 at $t=0$ h) as described (see Single Bolus Intracoronary FGF-2 Administration). However, in this simulation, a second bolus injection of exogenous FGF-2 was administered into the coronary circulation at $t=10$ h. The temporal results of this repetitive IC administration simulation are presented in Figs. 9 and 10. Figure 9A depicts the change in free interstitial FGF-2 concentration, and Fig. 9B shows the changes in the interstitial complex concentrations as a function of time. Figure 10, A and B (for endothelial cell) and C and D (for myocyte), depicts the changes in cell surface and internalized complex concentrations, respectively, as functions of time.

The concentrations of interstitial and cell-associated FGF-2 increased substantially almost immediately after the first and second injections. However, these concentration increases were transient as is illustrated by the sharp peaks and troughs in the temporal concentration plots. The myocardium then slowly returned to its basal state and reached within 5% of its basal state 21 h after the first injection and attained 1% of its basal state 28 h after the first injection.

Sensitivity Analysis

A comprehensive set of experimental data was analyzed to obtain experimentally determined parameter values that reflect the in vivo conditions of the canine myocardium. When such values were unavailable, a representative average value was selected based on the available experimental literature. To account for the uncertainty in these values, the sensitivity of the model results to the estimated parameter values was investigated.

The experimentally determined cell surface receptor concentrations are reported in units of receptors per cell. As a result, the effective cell surface area is an important determinant of cell surface receptor concentration. The effective cell surface area has been experimentally determined for cardiac myocytes but not for cardiac capillary endothelial cells. Therefore, the sensitivity of the model results to the assumed effective endothelial cell surface area (default value, 1,000 $\mu m^2$) was investigated.
An effective endothelial cell surface area of 500 \( \mu \text{m}^2 \) corresponds to two times the default total number of endothelial cell surface receptors (for FGFRs and HSPGs). The basal state simulation with low \( F_{0,\text{basal}} \) value was run for a total duration of 60 h. Similar to the default case, a fractional difference of \( <3.48 \times 10^{-7} \% \) was obtained between the steady-state concentrations obtained with all FGF-2 initially unbound and bound. Although the distribution of FGF-2 molecules within the myocardium was similar (19, 66, and 15\% of all FGF-2 molecules within the myocardium were found in the interstitium, internalized, and bound to the cell surfaces, respectively, compared with the respective default values of 28, 57, and 15\%), the total number of FGF-2 molecules bound to and internalized within the endothelial cells compared with the total number of FGF-2 molecules bound to and internalized within the myocytes was increased. With two times more FGFR and HSPG receptors on the endothelial cell surface, two times more \( FHR \) triads were formed on the endothelial cell surface (and, as a direct consequence, internalized within the endothelial cells). Because more FGF-2 ligands are bound to the endothelial cell receptors, fewer are available to bind to the myocyte receptors. Therefore, instead of the default 30:70\% endothelial-to-myocyte distribution of cell surface-bound FGF-2 molecules, an even 50:50\% distribution was obtained. Similarly, instead of the default 35:65\% endothelial-to-myocyte distribution of internalized FGF-2 molecules, a 58:42\% distribution was obtained.

An effective endothelial cell surface area of 1,500 \( \mu \text{m}^2 \) corresponds to 1.5 times fewer endothelial cell surface receptors (FGFRs and HSPGs). The basal state simulation with low \( F_{0,\text{basal}} \) value was run for a total duration of 60 h. In this case, fewer FGF-2 molecules were associated to the endothelial cells than to the myocytes. A distribution of 21:79\% endothelial-to-myocyte cell surface-bound FGF-2 molecules was obtained along with a distribution of 24:76\% endothelial-to-myocyte internalized FGF-2 molecules.

The sensitivity of the model results to effective endothelial cell surface areas of 500 and 1,500 \( \mu \text{m}^2 \) was also investigated.
using the basal simulation with high $F_{o,\text{basal}}$ value. The results from this simulation were qualitatively similar to those obtained with low $F_{o,\text{basal}}$ value (not shown).

In general, it appears that the effective endothelial cell surface area affects the distribution of FGF-2 molecules bound to or internalized within the endothelial and myocyte cells. However, it does not significantly alter the overall distribution of interstitial, cell surface, and internalized FGF-2 ligands within the myocardium. This study is concerned with the deposition and retention of FGF-2 within the myocardium after IC injection of endogenous FGF-2. Alternatively, experimental values for the number of cell surface receptors per unit area could be used; in this case, the cell surface area is not required.

The rate at which the luminal FGF-2 molecules cross the capillary walls and enter the interstitial space is regulated by the permeability value. A permeability of $12 \times 10^{-7}$ cm/s was assumed based on the experimentally determined permeability of swine coronary arterioles to $\alpha$-lactalbumin (34). The sensitivity of the model results to the permeation rate was investigated using permeability values ranging from $(10$ to $20) \times 10^{-7}$ cm/s.

The temporal results and steady states obtained using the basal state simulation with low and high $F_{o,\text{basal}}$ values were unaltered over the permeability range of $(10$ to $20) \times 10^{-7}$ cm/s. Similarly, owing to the already high endogenous level of FGF-2 within the interstitium of the myocardium, the temporal results obtained using the IC administration simulation with high $F_{o,\text{basal}}$ value were unaltered over the above-stated permeability range (flat curves).

The only significant change observed over the range of permeability values was in the maximum free interstitial FGF-2 concentration predicted from our IC administration simulation with low $F_{o,\text{basal}}$ value. Figure 11 depicts the change in free interstitial FGF-2 concentration as a function of time for permeability values of $10 \times 10^{-7}$, $12 \times 10^{-7}$ (default), and $20 \times 10^{-7}$ cm/s. The plots of interstitial, cell surface, and internalized complex concentrations as a function of time are not presented for the sake of brevity; they illustrate similar behavior to that depicted in Fig. 11. Note that as in the default IC administration simulation with low $F_{o,\text{basal}}$ value, most of the effects of the IC injection (observed with permeability values of $10 \times 10^{-7}$ and $20 \times 10^{-7}$ cm/s) occurred within the first 10 h. Moreover, the myocardium returned to within $2.57 \times 10^{-4}\%$ (with $P = 10 \times 10^{-7}$ cm/s) and $5.40 \times 10^{-4}\%$ (with $P = 20 \times 10^{-7}$ cm/s) of its basal state 60 h after the injection. The overall distribution of interstitial, cell surface, and internalized FGF-2 ligands was unaltered over the range of permeability values. However, the rate of FGF-2 permeation across the capillary wall did alter the myocardial uptake of exogenous FGF-2. The outer bounds of the permeability range considered here generate a difference of a factor of 1.6 in the maximum concentration of FGF-2 molecules found within the myocardium subsequent to the injection (as shown in Fig. 11). Although this difference is significant, it does not alter the overall qualitative model results. Assuming a low enough endogenous concentration of free interstitial FGF-2 to permit the permeation of additional FGF-2 molecules from the microvascular to the interstitial spaces, the IC administration of FGF-2 produces a transient increase in the concentrations of signaling complexes within the myocardium even at the lower bound of the permeability range. Additional experiments are required to determine the exact permeability value for FGF-2 through the myocardial capillary walls before quantitative conclusions can be drawn regarding this increase in signaling complexes.

**DISCUSSION**

**Model Predictions**

We have developed and implemented a compartmental model of the myocardium. This in vivo model was used to investigate the myocardial deposition and retention of FGF-2 at the cellular level after IC administration of FGF-2. A preliminary simulation was performed to determine the basal state of the myocardium (without administration of exogenous FGF-2). The IC administration of a single bolus of exogenous FGF-2 was then simulated using the results from the basal state simulation as the initial conditions (steady state). Finally, the
repetitive administration of identical injections of exogenous FGF-2 was simulated.

Owing to the uncertainty in the reported absolute concentration of endogenous FGF-2 present within heart tissue, two cases were considered: a low level of endogenous FGF-2 present within the myocardium, which resulted in a large concentration gradient between the microvascular and the interstitial spaces, and a high level of endogenous FGF-2 present within the myocardium, which resulted in a negligible concentration gradient between the microvascular and the interstitial spaces.

The model predictions from the basal state simulation suggested that the majority of the FGF-2 molecules found outside the cells (i.e., not internalized) are located within the interstitium, and that among the FGF-2 molecules found within the interstitium, almost all are bound to interstitial HSPGs. As a result, few FGF-2 ligands are free to bind to cell surface receptors. These results support the theory of extracellular control of the bioavailability of FGF-2 via dynamic storage of FGF-2 within the basement membranes and ECM.

The model predictions from the single bolus IC injection simulation in the case of a low level of endogenous FGF-2 present within the myocardium suggested that the luminal FGF-2 would rapidly permeate across the capillary walls and enter the interstitial space. A maximum interstitial FGF-2 concentration was reached ~20 min after the injection. As in the basal state, 98% of the interstitial FGF-2 ligands were bound to matrix HSPGs. Owing to the abundance of cell surface HSPGs, large \( F \) concentrations formed on the endothelial and myocyte cell surfaces subsequent to the injection. Many of these \( F \) complexes were subsequently converted into \( FHR \) triads, which led to large cell surface and internalized \( FHR \) concentrations. However, the increases in the interstitial and cell-associated FGF-2 concentrations as a result of the IC injection were transient and became negligible 10 h after the IC injection.

The model predictions from the single bolus IC injection simulation in the case of a high level of endogenous FGF-2 present within the myocardium suggested negligible changes in the myocardial FGF-2 ligand and complex concentrations as a
The permeation of additional FGF-2 molecules from the microvascular level of free interstitial FGF-2 is low enough to allow myocardial FGF-2 signaling complex concentration if the concentration of endogenous FGF-2 within the myocardial tissue.

Model Limitations

The model presented in this study is the first in vivo model developed to characterize the myocardial deposition and retention of FGF-2 at the cellular level after IC administration of FGF-2. Model limitations exist that should be addressed in future work.

Overall, the results obtained in this study suggest that the IC administration of FGF-2 causes a transient increase in the total myocardial FGF-2 signaling complex concentration if the endogenous level of free interstitial FGF-2 is low enough to allow the permeation of additional FGF-2 molecules from the microvascular to the interstitial spaces. However, our model does not consider the secondary effects of this administration of exogenous FGF-2. The uptake of FGF-2 into diseased tissue has been proposed to stimulate the expression of VEGF and other angiogenic growth factors, which may in turn lead to prolonged and sustained angiogenic activity (45). In particular, the upregulation of VEGF would quickly change the vascular permeability to FGF-2. Additional experimental studies are required to quantify the contributions of these other growth factors to the angiogenic activity that results from IC injection of FGF-2.

Our model parameter values were based on experimental data gathered from a number of different species. An attempt was made to use canine experimental data whenever possible. When such values were unavailable, a cross section of experimental values obtained for a variety of different species was collected, and a representative average value was selected. It is understood that various species may react differently to concentrations of endogenous and exogenous FGF-2. Future models should incorporate more canine experimental data as they become available.

Additionally, owing to a lack of experimental data on the diseased myocardium, the parameter values used in this study reflect the healthy myocardium. In reality, the morphology of diseased tissue is different than that of healthy tissue. A diseased myocardium may have fewer perfused capillaries; the basement membranes may be thicker; and the cell surface receptor concentrations and luminal FGF-2 ligand concentrations may be upregulated in response to the ischemic conditions. It is unknown how much of an effect these morphological changes would have on the overall angiogenic activity resulting from IC injection of FGF-2. Additional experimental studies should be conducted to characterize the properties of the diseased myocardium.

The approach to analysis of complex systems, like the one considered here, is to develop computational models in a modular fashion rather than to attempt formulation of a comprehensive model containing hundreds of parameters, because it would be impossible to validate the model and understand the results. The present analysis is a necessary step toward developing a more complete quantitative picture of FGF-2-induced angiogenesis. We expect that the present model would serve as a module in a more general model that would include signal transduction and release of FGF-2 from the ECM. Models of ligand-receptor interactions are being developed as independent modules for several growth factor families. These include models of FGF-FGFR interactions (8, 18, 19, 22, 25, 26, 31, 35), VEGF-VEGF receptor interactions (58a), and EGF-EGF receptor interactions (44, 71). Computational modeling of downstream receptor signaling for these systems requires a large amount of experimental data on the dynamics of signaling molecules. Such models have thus far only been developed for the EGFR system (43, 59, 74) and platelet-derived growth factor (68). Modeling FGFR signaling would constitute a major experimental/computational project and would certainly be a worthwhile addition to a general FGF-2 model.

Finally, computational models of release of growth factors from the ECM would require a separate theoretical effort and experimental data on the interactions of ECM-bound growth factors, e.g., with the metalloproteinase- and plasminogen-
activator systems. Work in this area of ECM proteolysis with metalloproteinases has recently begun (39).

APPENDIX A

Parameter Values and Related Assumptions

To investigate the rate of FGF-2 diffusion within the interstitium, diffusion distances and diffusivity values are required. The EBM and MBM are similar in size and composition (21). Based on this observation, the physical properties of the modeled EBMs and MBMs are assumed identical. The ECM, however, is much thicker and less dense than the basement membranes (3, 14).

No experimental values for canine basement membrane or ECM thickness were reported. Nevertheless, the thickness of healthy human cardiac capillary membranes and MBMs was found to vary from 30 to 50 nm, with 50 nm considered normal (21, 76). Intermyocyte spacing values (which are assumed equal to the distance between capillaries and myocytes) of 1.8 and 2.7 \( \mu \)m were also reported for rabbit (30, 41) and guinea pig (6) hearts, respectively. Based on these experimental results, a basement membrane thickness of 50 nm was assumed along with an endothelial cell-to-myocyte spacing of 2 \( \mu \)m, which provides an ECM thickness of 1.9 \( \mu \)m.

The diffusivity of FGF-2 within the basement membranes and ECM differs because of dissimilarities in matrix composition and density. Descemet’s membrane is commonly used as a representative model of in vivo basement membranes. Thus the diffusivity of FGF-2 within the EBMs and MBMs was assumed equal to \( 6 \times 10^{-6} \) cm\(^2\)/s, which corresponds to the reported experimentally determined diffusivity value for FGF-2 in Descemet’s membrane (14). The diffusivity of FGF-2 within the ECM, meanwhile, has not been experimentally determined. An estimated value of \( 2.11 \times 10^{-5} \) cm\(^2\)/s was used; this was obtained based on the molecular size of FGF-2 and the diffusive hindrance that results from the composition of the ECM. A detailed explanation of this estimate is presented in APPENDIX B.

Several conversion factors were used to convert the reported concentration values into units of molecules per volume of myocardial tissue. In particular, the volume fractions that correspond to the microvascular, interstitial, and parenchymal cell spaces within the myocardium were used to convert units of molecules per volume of fluid into units of molecules per volume of myocardial tissue. A study on guinea pig hearts reported volume fractions of 0.042, 0.220, and 0.738 ml/ml of myocardial tissue for the microvascular, interstitial, and parenchymal cell spaces, respectively (6). A study on rabbit hearts reported volume fractions of 0.064, 0.234, and 0.617 ml/ml of myocardial tissue volume fractions from units of molecules per cell surface area to molecules per volume of myocardial tissue. Capillary luminal diameters have been reported in the range of 4–6 \( \mu \)m (5, 6, 29, 41, 42, 60, 61, 69). Assuming a capillary wall thickness of 0.5 \( \mu \)m and an average luminal diameter of 5 \( \mu \)m, the abluminal diameter of all modeled capillaries was set to 6 \( \mu \)m. With the use of this capillary abluminal diameter and the microvascular space volume fraction (described above), an endothelial cell surface area-to-tissue volume fraction of 509 \( \text{cm}^2/\text{cm}^3 \) was obtained. Similarly, using a cardiac muscle fiber radius of 12.5 \( \mu \)m and the parenchymal space volume fraction, a myocyte cell surface area-to-tissue volume fraction of 1.085 \( \text{cm}^2/\text{cm}^3 \) was obtained.

Typically, basement membranes have a much larger concentration of HSPG binding sites than their associated ECM (3, 14). Although HSPG binding site concentrations found within canine MBMs and ECM have not been reported, an HSPG binding-site concentration of 13 \( \mu \)M was reported for Descemet’s membrane (14). An HSPG binding-site concentration of 0.75 \( \mu \)M was also reported for the ECM produced by cultured endothelial cells (3). As in previous models (22, 26), we assume one FGF-2 binding site per HSPG molecule. The HSPG concentrations within the modeled basement membranes and ECM were therefore set to 13 and 0.75 \( \mu \)M, respectively.

Uniform association and dissociation rate constants were assumed for FGF-2 interactions with interstitial HSPGs. The \( k_{a0} \) and \( k_{d0} \) values of 25.2 \( \mu \)M\(^{-1}\) min\(^{-1} \) and 0.6 min\(^{-1} \) were assumed, which correspond to the experimentally determined association and dissociation rates for FGF-2 within Descemet’s membrane at 37°C (14). The kinetic rate constants for the endothelial and myocyte cell surfaces (association, dissociation, internalization, degradation, and receptor production), meanwhile, were set to the values reported in our previous in vitro model at 37°C (22). These kinetic rate constants were either experimentally determined or estimated based on the available experimental literature.

FGF-2 molecules circulate endogenously in the blood. Owing to their small size, the FGF-2 molecules are presumed to travel almost exclusively between the microvasculature and the interstitium via their small size, the FGF-2 molecules are presumed to travel almost exclusively between the microvasculature and the interstitium via the endothelial intercellular clefts (4, 27). This behavior is represented in our model by an FGF-2 permeation rate across the capillary wall, which is given by

\[
p_P = P \times ([F]_L - [F])
\]

(A1)

where \( p_P \) is the permeation rate, \( P \) is the capillary wall permeability, and \( ([F]_L - [F]) \) is the FGF-2 concentration gradient within the intercellular cleft.

It is important to note that vascular FGF-2 molecules can bind to some of the blood components including soluble HSPGs and proteins. However, the kinetics of these interactions have been poorly characterized. As a result, these interactions are not included in this model.

The permeability of small blood vessels to FGF-2 has not been reported. However, several studies have reported microvascular permeability values for \( \alpha \)-lactalbumin (a globular, 14-kDa protein with Stokes radius of 2.01 nm), which is a solute of comparable size to FGF-2 molecules. A study using rat mesenteric capillaries reported an \( \alpha \)-lactalbumin permeability of 55 \( \times 10^{-7} \) cm/s (27), whereas another study that also used frog mesenteric capillaries reported an \( \alpha \)-lactalbumin permeability ranging from 21 \( \times 10^{-7} \) to 40 \( \times 10^{-7} \) cm/s (33). A study using rat mesenteric capillaries reported an \( \alpha \)-lactalbumin permeability of 50 \( \times 10^{-7} \) cm/s (86). Another study using swine coronary arterioles reported a mean \( \alpha \)-lactalbumin permeability of 12 \( \times 10^{-7} \) cm/s (34). The close agreement between permeability values reported for the frog and rat mesenteric capillaries suggests consistent permeability values across various species. However, the mesenteric circulation is
not an ideal model for the myocardial microvasculature. The permeability of the modeled myocardial capillaries to FGF-2 was therefore assumed equal to $12 \times 10^{-7}$ cm/s based on the reported permeability of swine coronary arterioles to $\alpha$-lactalbumin. A sensitivity analysis was performed with permeability values in the range of $(10$ to $20) \times 10^{-7}$ cm/s (discussed in Sensitivity Analysis).

The level of endogenous FGF-2 within the plasma of healthy human volunteers is quite low. One study reported undetectable levels of plasma FGF-2 in all their healthy human subjects (15), whereas another study reported levels of plasma FGF-2 between $4.4 \times 10^{-8}$ and $1.4 \times 10^{-6}$ M (66). Based on these experimental results, a constant basal luminal FGF-2 concentration of $4.4 \times 10^{-8}$ was assumed.

The secretion of FGF-2 from cardiac endothelial cells and myocytes is known to occur (12, 23, 75); nevertheless, experimental secretion rates for these cells have not been reported. The FGF-2 secretion rates for the modeled endothelial cells and myocytes (assumed equal) are therefore set such that the degradation of internalized FGF-2 is matched by the secretion of new FGF-2 ligands (and the permeation of additional FGF-2 into the interstitial space from the microvascular space, if a sufficient concentration gradient exists). In this way, a constant basal level of FGF-2 is maintained within the myocardial tissue. Secretion rates of $7.20 \times 10^{-11}$ and $1.08 \times 10^{-11}$ mmol/cm$^2$-min were used for constant FGF-2 basal levels of 6 and 228.5 $\mu$g/kg of tissue, respectively. Note that smooth muscle cells and fibroblasts are also present within the myocardium, and they also secrete FGF-2 monomers into the interstitial space (12, 13). Because all parenchymal cells within the modeled myocardium are assumed to be myocytes, the secretion of FGF-2 from smooth muscle cells and fibroblasts is indirectly considered.

The HSPGs within the interstitium and the FGFRs and HSPGs on the cell surfaces were assumed initially to be unbound (no preformed complexes). The initial endothelial cell surface FGFGR and HSPG concentrations were set to $2.5 \times 10^{10}$ and $1.3 \times 10^5$ receptors/cell, respectively, which correspond to the experimentally determined concentrations for cardiac microvascular endothelial cells (55). No experimental values have been reported for the receptor concentrations on cardiac myocytes. Therefore, the initial myocyte cell surface FGFGR and HSPG concentrations were set to the general, phenotypically relevant values of $10^4$ and $10^6$ receptors/cell, respectively (37, 72).

**APPENDIX B**

**Estimation of FGF-2 Diffusivity Within the Extracellular Matrix**

The myocardial interstitium is a complex assembly of collagen, glycosaminoglycans (GAGs), and proteoglycans, which hinders the diffusion of FGF-2. To account for the diffusive hindrance resulting from the composition of the ECM, the diffusivity of FGF-2 in the myocardial ECM was estimated using

$$D = D_v \times \exp\left(-\frac{k_{\text{diff}} r_e^2}{r_f^2}\right)$$

(B1)

proposed by Ogston et al. (65) and discussed by Johnson et al. (38) to describe the diffusion of spherical molecules (such as FGF-2) with radius $r_e$ through solutions of linear polymers. This expression is formulated for a randomly oriented array of straight, cylindrical fibers of radius $r_f$ and fiber volume fraction $\phi$. $D$ and $D_v$ are the diffusivities of FGF-2 in the polymer solution and in free solution, respectively.

The molecular radius of FGF-2 is 1.45 nm, and the aqueous diffusivity of FGF-2 at 37°C is $2.30 \times 10^{-7}$ cm$^2$/s (22). The myocardial ECM is composed of $14$ mg/g of interstitium of collagen and $1.2$ mg/g of interstitium of GAG chains (54). Assuming that most of the diffusive hindrance is due to the collagen molecules, with a fiber radius of 20 nm and a fiber volume fraction of 0.026 (54), we obtain an estimated diffusivity for FGF-2 in the ECM of $2.27 \times 10^{-7}$ cm$^2$/s. Alternatively, assuming that most of the diffusive hindrance is due to the GAG chains, with a fiber radius of 0.55 nm and a fiber volume fraction of $7.8 \times 10^{-4}$ (54), we obtain an estimated diffusivity for FGF-2 in the ECM of $2.14 \times 10^{-7}$ cm$^2$/s. Because the diffusive hindrance is probably due to the combined effects of the collagen and GAG fibers present within the ECM, a combined FGF-2 diffusivity value is used, which is calculated according to

$$D = D_v \times \exp\left(-\frac{k_{\text{diff}} r_e^2}{r_f^2}\right) \times \exp\left(-\frac{k_{\text{diff}} r_e^2}{r_G^2}\right)$$

(B2)

and equals to $2.11 \times 10^{-7}$ cm$^2$/s. Note that this diffusivity value is 35 times faster than the experimentally determined diffusivity value for FGF-2 in the EBMs and MBMs, which reflects the less-dense composition of the ECM compared with the basement membranes.

**APPENDIX C**

**Model Justification**

The Damkohler number, which is defined as $D_a = \frac{k_{\text{diff}} L^2}{D}$, where $k_{\text{diff}}$ is the dissociation rate, $L$ is the diffusion distance, and $D$ is the diffusivity, is often used to determine whether a particular system is reaction limited ($D_a << 1$) or diffusion limited ($D_a >> 1$). Assuming an average capillary-to-myocyte (and myocyte-to-myocyte) diffusion distance of 2 $\mu$m, Damkohler numbers of 0.067 (basement membrane diffusivity) and 0.0019 (ECM diffusivity) are obtained for the diffusion of FGF-2 through the interstitium compared with the dissociation of FGF-2 from an interstitial HSPG. Based on these values, we infer that the rate of FGF-2 diffusion through the interstitium is much faster than the rate of FGF-2 reaction with interstitial HSPGs. A similar analysis can be performed for the rate of FGF-2 diffusion through the interstitium compared with the rate of FGF-2 reaction with its endothelial and myocyte cell surface receptors: using an average diffusivity value of $1.09 \times 10^{-7}$ cm$^2$/s and an average dissociation rate of 0.733 min$^{-1}$, we obtain a Damkohler number of 0.0045. Note that the different combinations of dissociation rates and diffusivity values produce a range of Damkohler numbers between 0.00012 and 0.15 (all much << 1). Thus the diffusion rate of FGF-2 through the interstitium is much faster than the reaction rate of FGF-2 with its cell surface receptors. Overall, our system is reaction limited. It is therefore reasonable to neglect the spatial gradients of free FGF-2 ligand for the purpose of the present analysis.

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