Macromolecular transport in heart valves. II. Theoretical models

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Zeng Z, Yin Y, Jan K-M, Rumschitzki DS. Macromolecular transport in heart valves. II. Theoretical models. Am J Physiol Heart Circ Physiol 292: H2671–H2686, 2007. First published January 12, 2007; doi:10.1152/ajpheart.00608.2006.—This paper proposes a new, two-dimensional convection-diffusion model for macromolecular transport in heart valves based on horseradish peroxidase (HRP) experiments on rats presented in the first of the papers in this series (Part I; Zeng Z, Yin Y, Huang AL, Jan KM, Rumschitzki DS. Am J Physiol Heart Circ Physiol 292: H2664–H2670, 2007). Experiments require two valvular intimae, one underneath each endothelium. Tompkins et al. (Tompkins RG, Schnitzer JJ, Yarmush ML. Circ Res 64: 1213–1223, 1989) found large variations in shape and magnitude in transvalvular 125I-labeled low-density lipoprotein (LDL) profiles from identical experiments on four squirrel monkeys. Their one-dimensional, uniform-medium diffusion-only model fit three parameters independently for each profile; data variability resulted in large parameter spreads. Our theory aims to explain their data with one parameter set. It uses measured parameters and some aortic values but fits the endothelial mass transfer coefficient ($k_e = 1.63 \times 10^{-8}$ cm/s, where subscripts $a$ and $v$ indicate aortic aspect and ventricular aspect, respectively) and middle layer permeability ($K_p = 2.28 \times 10^{-16}$ cm$^2$ and LDL diffusion coefficient $D_L(LDL) = 5.93 \times 10^{-9}$ cm$^2$/s), using one of Tompkins et al.’s profiles, and fixes them throughout. It accurately predicts Part I’s rapid localized HRP leakage spot growth rate in rat leaflets that results from the intima’s much sparser structure, dictating its far larger transport parameters ($K_p = 1.10 \times 10^{-12}$ cm$^2$, $D_L(LDL/HRP) = 1.02 \times 4.09 \times 10^{-7}$ cm$^2$/s) than the middle layer. This contrasts with large arteries with similarly large HRP spots, since the valve has no internal elastic lamina. The model quantitatively explains all of Tompkins et al.’s monkey profiles with these same parameters. Different numbers and locations of isolated macromolecular leaks on both aspects and different section-leak(s) distances yield all profiles.

Convection-diffusion; two-dimensional transport; variation in LDL profiles

MACROMOLECULAR TRANSPORT, especially that of low-density lipoprotein (LDL), in heart valves plays an important role in the initial prelesion steps of valves subject to tissue calcification, sclerosis, and stenosis (22, 24–26, 29, 38, 39). Tompkins et al. (34) performed the first, and thus far only, detailed measurements of such transport by using quantitative autoradiography to examine the transvalvular 125I-LDL concentration profiles in the aortic valves of squirrel monkeys as a function of the depth into the leaflet after 30-min 125I-LDL circulation in vivo (see Fig. 1). Their simple, one-dimensional (1D) mathematical analysis of these observations implicitly assumed that 1) the transport process in heart valves depends only on the direction normal to the endothelial surface, 2) molecular diffusion is the only important transport mechanism, and 3) one can treat the matrix interposed between the aortic and ventricular aspects of the leaflet as an isotropic, uniform medium. They then fit each of their experimental profiles to the model to get a set of three transport parameters, an effective diffusion coefficient in the tissue and two endothelial mass transfer coefficients at the aortic and ventricular aspects of the aortic valve, for each profile. Unfortunately, the magnitudes and even the shapes of the measured transvalvular concentration profiles among different regions examined were very different and, consequently, the parameters fitted for the different profiles exhibited very large variations of up to 10 orders of magnitude, but more typically by factors of 10–20. This large variation appeared to signify a large variability in the mass transport between different leaflets of the same aortic valve and even between different regions of the same leaflet. It thus appears that this 1D model is, to some extent, too simple and is meant to quantify, rather than to account for, these large variations (46).

Previous studies (8, 27, 31) revealed that molecules or molecular aggregates bigger than albumin (~7 nm) largely cross the endothelial and transport into the walls of blood vessels by passing through transient, localized endothelial leaks, rather than uniformly, and spread rapidly in the subendothelial intima. These leaks are often attributable to widened junctions of endothelial cells (ECs) that are either dying or dividing (8, 15, 19–21). This so-called leaky junction-cell turnover hypothesis suggested that a tiny fraction of leaky vascular ECs could account for the experimentally observed local variations in endothelial macromolecular permeability in large arteries (19). Motivated by this earlier work in vessels (8), our experimental studies with horseradish peroxidase (HRP) in the first of the papers in this series (Ref. 46; Part I, this issue) sought to discover whether macromolecular leakage into the valve was similar. En face interrogation under light microscopy of aortic valve leaflets from normal Sprague-Dawley rats after short-time HRP circulation showed only very few, localized HRP leaks, seen as isolated brown spots in the endothelium after histochemical treatment (Fig. 1 in Ref. 46), rather than a uniform brown staining. For rats, this contradicts the earlier model’s (34) first assumption and indicates that macromolecular transport in heart valves depends on the direction parallel to, as well as the direction normal to, the endothelium, i.e., transport is at least two-dimensional (2D).

Part I also examined the growth of these HRP leakage spots with increasing HRP circulation time (Fig. 2 in Ref. 46). This growth is extremely fast, from roughly 12- to almost 50-μm radius in 1 min [of which ~25 s was needed for the tracer to reach the aorta (16) or valve (see Fig. 10) from the point of...
Fig. 1. Tompkins et al.’s low-density lipoprotein (LDL) transvalvular concentration profiles after 30-min in vivo circulation in 4 squirrel monkeys. The y-axis is the volumetric tissue concentration ($C_T$) divided by the plasma concentration at 5 min ($C_{p0}$). The x-axis is the depth into the valve ($X$) divided by the width or thickness of the leaflet sections ($L$), measured from the aortic aspect, $X/L = 0$, to the ventricular aspect, $X/L = 1$, of the aortic valvular leaflet. $n$, Number of individual profiles averaged to generate the profiles shown. Smooth curves are the parameter optimization in Tompkins et al.’s 1-dimensional diffusion model. [From Tompkins RG, Schnitzer JJ, Yarmush ML. Macromolecular transport within heart valves. Circ Res 64: (1213–1223, 1989, with permission (34)].

infusion in the femoral vein] and to 75 μm in 4 min. In fact, considering that the valve leaflet is closed, and thus subject to a transverse pressure gradient, for only 60–70% of the cardiac cycle, the spot growth is comparable to that in the aorta. It is unlikely that molecular diffusion alone can account for it without an unrealistically high diffusivity (at least $\sim 10^{-6}$ cm$^2$/s). Hence, the model should allow for convection, to see whether it is indeed significant, as it turns out to be in large arteries (16). The different lumens pressures on the endothela of the leaflet’s aortic and ventricular aspects when the aortic valve is closed would provide the driving pressure for convection across the valve. The difference in the hydraulic conductance of the (rare) leaky and the (vastly more numerous) normal EC junctions would result in a subendothelial pressure gradient. This gradient parallel to the endothelium could drive a convection that could account for the rapid growth of HRP leaky spots.

Finally, transmission electron microscopic examination of traverse sections of rat valve leaflets after 4-min HRP circulation showed a very thin, $\sim 150$-nm, region of intense staining, followed by a step change to much more diffuse staining. This likely indicates the existence of a sparse layer immediately beneath the valve’s endothelia where the HRP concentration is much higher than that in the matrix below it (Fig. 3 in Ref. 46). We called this layer the “valvular subendothelial intima,” in analogy to the vascular subendothelial intima, and the balance of the interposed matrix the “middle layer.” Nievelstein-Post et al.’s (22) electron micrograph of an ultrarapid freezing/rotary shadow etching of a normal rabbit valve’s subendothelial space showed a clear step change in matrix porosity from a sparse, immediately subendothelial region of the same dimension to a much thicker, dense region. The much sparser structure of the valvular intima allows for a much larger available volume for fluid and macromolecules. In particular, the typical spacing between the dominant matrix fibers and collagen in the valve intima is $\sim 30–40$ nm in Fig. 4 of Ref. 46, which is the same as that in the aortic intima (11, 16, 23). The fiber matrix theory that these references expound leads to ab initio calculated values for the intima transport parameters that are the same as those in the aortic intima. As we show here, even in the presence of convection, the existence of sparse intima regions is necessary to account for the observed rapid growth of HRP leaky spots.

The new experimental results in Part I (46) guide us to advance a 2D convection-diffusion model in which the valvular intimae are much thinner and sparser than the balance of the interposed matrix. We use this model to explain the rapid growth of HRP spots in rat valves noted above. Whereas this model is based on rat (and some rabbit) valve data, we apply it to the monkey valve as well in an attempt to explain the observed large variations in Tompkins et al.’s transvalvular LDL concentration profiles in Fig. 1. We suggest that this variation might have resulted from the different number and position of leaky ECs on the valve’s endothelia and from the position of the tissue sections examined relative to those leaks. Rather than using a very different set of parameters for each transvalvular LDL concentration profile as in Ref. 34, we use a single parameter set to rationally explain all of Fig. 1’s experimental monkey profiles. The close agreement between theory and experiment for these profiles and for the HRP spot growth (46) strongly supports our model.

**MATHEMATICAL MODELS**

**Glossary**

- $Bi_j$: Biot number at the aortic aspect, $j = a$, and the ventricular aspect, $j = v$
- $C_i, C_i^*$: Solute concentration locally averaged over all phases locally present in layer $i$ as a function of position and time
- $C_l, C_l^*$: Solute concentration in the leaky junction
- $C_c$: Critical (minimum detectable) HRP concentration
- $C_p$: Solute concentration in plasma
- $D_{i}, D_{i}^*$: Diffusion coefficient in layer $i$
- $D_{l}^*$: Diffusion coefficient in the leaky junction
- $f_i$: Retardation coefficient in layer $i$
- $f_{l}^*$: Retardation coefficient in the leaky junction
**Superscript and Subscripts**

- \( k_i \): Endothelial mass transfer coefficient at the aortic (\( j = a \)) and ventricular (\( j = v \)) aspects.

- \( K_{pi}, K_{pi}^* \): Permeability in layer \( i \).

- \( L \): Thickness of valve leaflet.

- \( L_i \): Thickness of layer \( i \).

- \( L_j \): Length of leaky junction.

- \( L_{pi}^* \): Hydraulic conductivity in the middle layer.

- \( L_{pi}, L_{pi}^* \): Hydraulic conductivity of the leaky junction at the aortic (\( j = a \)) and the ventricular (\( j = v \)) aspects.

- \( L_{pi}, L_{pi}^* \): Hydraulic conductivity of the normal junction at the aortic aspect, \( j = a \), and the ventricular aspect, \( j = v \).

- \( \text{Pc}_{ir} \): Radial Péclet number in layer \( i \).

- \( \text{Pc}_{iz} \): Normal Péclet number in layer \( i \).

- \( \text{Pc}_{j} \): Péclet number in the leaky junction.

- \( P_i \): Pressure in layer \( i \).

- \( P_i^* \): Lumen pressure at the aortic aspect, \( j = a \), and the ventricular aspect, \( j = v \).

- \( q_i, q_i^* \): Solute flux in the leaky junction.

- \( q_i \): Solute flux vector in layer \( i \).

- \( q_{ir}, q_{ir}^* \): Radial solute flux in layer \( i \).

- \( q_{iz}, q_{iz}^* \): Normal solute flux in layer \( i \).

- \( r, r^* \): Radial distance or direction parallel to the endothelium.

- \( R_1 \): Radius of the endothelial cell, i.e., inner radius of the leaky cleft.

- \( R_2 \): Outer radius of the leaky cleft.

- \( U_i, U_i^* \): Radial velocity in layer \( i \).

- \( V_i \): Velocity vector in layer \( i \).

- \( W_i, W_i^* \): Normal velocity in layer \( i \).

- \( x \): Length dimension in the leaky junction.

- \( z, z^* \): Normal distance or direction perpendicular to the endothelium.

- \( \gamma_i \): Volume fraction available to solute in layer \( i \).

- \( \mu \): Fluid viscosity.

- \( \tau, t \): Time.

- \( t_0 \): Time it takes injected HRP to reach aortic valve.

- \( \xi \): Radius of the model unit.

- \( \Delta P^* \): Lumen pressure difference between the aortic and the ventricular aspect of the aortic valve.

- \( \Delta R \): Width of the leaky junction.

**Model description.** Figure 2 illustrates the proposed aortic valve model. It is comprised of two endothe-lias, defining the aortic and ventricular aspects. Each endothelium contacts the lumen on the outside and a thin intima on the inside. A thick middle matrix layer lies between the two intimae. This sometimes mentioned (14, 28) but seldom emphasized valve intima (thickened when diseased; Refs. 6, 24) plays a critical role in short-time macromolecular transport in heart valves. Each of these matrix layers is assumed to be a uniform, isotropic, homogeneous, and porous medium. Since we have no reason to assume otherwise, we assume that the two valve intimae are morphologically and histologically identical.

A rat aortic valvular leaflet has an area of \( \sim 1 \text{ mm}^2 \) covered with ECs of an average radius of 11.7 \( \mu \text{m} \) (46), i.e., \( \sim 2,300 \) ECs/surface. In the most lesion-susceptible regions of the rat aorta, approximately 1 in 2,000–6,000 ECs (19) (\( \sim 1/5,000 \)) in rabbit; Ref. 1) has a junction that leaks at any given time. If the valve leaflet’s leakage frequency is similar, one expects \( \approx 0–2 \) leaks per aspect at any time, consistent with Part I’s (46) HRP spot frequency in rat valves of 0–3 leaks per leaflet. We

![Fig. 2. Schematic illustration of the models of the valve leaflet (A) and the leaky junction (B). The dimensions are not to scale so as to illustrate all of the regions. NEC and LEC, normal and leaky endothelial cells, respectively; \( r \), direction parallel to the endothelium; \( z \), direction normal to the endothelium; \( L_1, L_2 \), thickness of subendothelial intima at the aortic aspect and ventricular aspect, respectively; \( L_3 \), thickness of middle layer; \( R_1, R_2 \), inner and outer radius of leaky junction, respectively; \( x \), direction along leaky junction. C: 3 cases of LEC (black spot) locations on the semilunar aortic valve leaflet with the free edge on top: 1, leak at or near the center; 2, 2 leaks; 3, 1 leak close to the edge.](http://ajpheart.physiology.org/)

**Fig. 2.** Schematic illustration of the models of the valve leaflet (A) and the leaky junction (B). The dimensions are not to scale so as to illustrate all of the regions. NEC and LEC, normal and leaky endothelial cells, respectively; \( r \), direction parallel to the endothelium; \( z \), direction normal to the endothelium; \( L_1, L_2 \), thickness of subendothelial intima at the aortic aspect and ventricular aspect, respectively; \( L_3 \), thickness of middle layer; \( R_1, R_2 \), inner and outer radius of leaky junction, respectively; \( x \), direction along leaky junction. C: 3 cases of LEC (black spot) locations on the semilunar aortic valve leaflet with the free edge on top: 1, leak at or near the center; 2, 2 leaks; 3, 1 leak close to the edge.
assume that these leaky cells can occur on the endothelia of both aspects, and Part I found these leaks more likely to occur near the line of coaptation. That the leakage frequency estimate is consistent with observation is interesting, but since modeling HRP spot growth only assumes the existence of a leak, the leakage frequency and location do not enter as long as leaks are not too close together (see next two paragraphs). Similarly, since the purpose of our application of the model to the monkey data is to present a plausible explanation of what may have occurred in Tompkins et al.’s (34) specific measurements and not an average behavior over a large number of measurements, the leakage frequency and distribution are, again, not germane.

The model in Fig. 2 presumes that the local thickness of the leaflet does not change appreciably over distances of several EC radii, so that one can take the endothelia to be parallel. The model is based on an axisymmetric cylindrical slab of radius \( R_1 \) and height \( L \), the valvular leaflet’s local thickness. This cylindrical assumption encompasses three cases (Fig. 2C), each with a different region assumed to be circular. 1) If there is a single leak located at or near the center of the aspect, the entire leaflet is assumed circular; \( \xi \) is then the leaflet radius, \(-564 \mu m\). 2) If there is more than one leak on an aspect, we divide the leaflet surface into (by abuse of geometry) circular periodic units whose area equals that of the leaflet surface divided by the instantaneous number of leaks, e.g., \( \xi \approx 400 \mu m\) for two leaks in an aspect. The spacing between adjacent leaks is \( 2\xi \), as in arteries (16, 19, 37, 45). 3) If the leaf is far from the leaflet’s center, the circular region surrounds the leaflet and extends to the edge of the leaflet, a distance \( \xi \) away. As shown below, leafy junction influence on transport extends less than \(-15R_1, \approx 175 \mu m\) in the direction parallel to the endothelium; thus the solutions are insensitive to the precise \( \xi \) value when \( \xi > 175 \mu m\).

If the leaflet has no leaks, the problem reduces to one dimensional in \( z \) normal to the endothelial surface from the aortic \( z = 0 \) to the ventricular aspect \( z = L \), the local leaflet thickness. With \( \geq 1 \) leak, the problem becomes 2D with dependence on \( r \), parallel to the endothelial surface, from the center of the leaky cell to \( \xi \). Axisymmetry requires simultaneous leaks on both aspects that are close in \( r \) to both be located at \( r = 0 \). If they are far apart in \( r \), their interaction is minimal and we can superimpose two single-leak solutions. Although this violates randomness, ventricular aspect leaks where convection opposes diffusion are far less important than aortic leaks where it reinforces diffusion.

Filtration model. Over the cardiac cycle, the aortic valve periodically opens when the pressure in the left ventricle is higher than that in the aorta and closes otherwise. The pressure on the aortic valve leaflet’s ventricular aspect is negligibly higher than on its aortic aspect when the valve is open (30–40% of the time), but is much lower (~90 mmHg) when the valve is closed. As a simplification, we assume a steady, nonzero, time-averaged pressure difference of \( \Delta P^* \approx 50 \text{ mmHg} \), below the time averaged aortic transmural pressure of ~100 mmHg between the two aspects. This gradient drives the overall convection through the leaflet from the high-pressure aorta toward the lower-pressure ventricle. Water enters or leaves the leaflet through both normal and leaky endothelial junctions. The pressure drop across the leaky junction is much smaller than across the normal junction since its hydraulic conductivity \( (L_p^*) \) is much larger. Thus the pressure near the leaky junction is higher than that far from the leaky junction in the aortic aspect’s intima, and vice versa in the ventricular aspect’s intima. These radial pressure gradients and the intima’s relatively large permeability drive radial inflow, giving big spots.

\[ U^* \text{ (radial velocity) and } W^* \text{ (normal velocity)} \ [ V^* = (U^*, \ W^*) ] \] represent the local volume-averaged superficial water velocities, equal to the fluid’s volumetric flow rate divided by the total cross-sectional area including the area of the fluid and the tissue. \( V^* \) is governed by Darcy’s law, in \( r \) and \( z \). \( L_p^* \) and \( K_p^* \) are the hydraulic conductivity and permeability, each carrying a subscript denoting the region the parameters represent. The subscripts \( v \) and \( a \) represent the ventricular and the aortic aspects. The subscripts \( n \) (\( j = a, v \)) and \( l \) (\( j = a, v \)) represent the normal and leaky junctions at both the aortic and the ventricular aspects. \( L_p^*, \) is the homogenized, area-averaged value representing both normal junctions and cells. We shall be agnostic as to whether this solvent transport is purely around the ECs or through them as well, and thus assign the same value to the cell \( r < R_1 \), where \( R_1 \) is the average EC radius. (This complex topic is the subject of a forthcoming detailed experimental/theoretical investigation.) Clarification of possible transcellular flow may reduce the endothelial \( L_p^* \) for \( r < R_1 \), but since the area fraction and \( L_p^*/L_p^* \) are negligible, its water flux and influence on the problem are also negligible (see Fig. 11, curve 2a, for verification). A similar argument pertains to the mass transfer problem and, in particular, to Eqs. 20b and 22b below.

The subscript \( i \) denotes the aortic aspect’s intima (\( i = 1 \)), the middle layer (\( i = 2 \)), and the ventricular aspect’s intima (\( i = 3 \)). \( \mu \) is the fluid’s viscosity. \( \Delta P^* \) is the difference between the lumen \( (P^*_L) \) and ventricular \( (P^*_v) \) pressures. One symbol represents the same dimensional (with \( * \)) and nondimensional (without \( * \)) variable. The coordinates are normalized by the average thickness, \( L \), of the leaflet

\[ r = \frac{r^*}{L}, \quad z = \frac{z^*}{L} \quad (1) \]

We normalize the water velocities \( U^* \) and \( W^* \), pressures \( P^* \), hydraulic conductivities \( L_p^* \) and \( K_p^* \), and the Darcy permeability \( K_p^* \), in terms of the middle layer’s hydraulic conductivity \( L_p^* \), its permeability \( K_p^* \), and the pressure difference \( \Delta P^* \) as follows:

\[ U_i = \frac{U_i^*}{L_p^* \Delta P^*}, \quad W_i = \frac{W_i^*}{L_p^* \Delta P^*}, \quad i = 1, 2, 3 \quad (2) \]

\[ P_i = \frac{P_i^* - P_{i-1}^*}{\Delta P^*}, \quad K_p = \frac{K_p^*}{L_{p_{i-1}}}, \quad L_{p_i} = L_{p_{i-1}} \quad j = a, v \quad (3) \]

where

\[ L_p^* = \frac{K_p^* \mu L}{\rho L} \]

and

\[ K_p^* = 1 \]

Each porous matrix layer satisfies continuity, constant fluid mass density, and Darcy’s law:

\[ \frac{\partial}{\partial t} \int \rho \, \text{d}V + \int \rho \left[ \frac{\partial u}{\partial t} + u \cdot \nabla u \right] \, \text{d}V = \int \ell \cdot \nabla \cdot \nabla u \, \text{d}V \]

\[ \int \rho \left[ \frac{\partial u}{\partial t} + u \cdot \nabla u \right] \, \text{d}V = \int \ell \cdot \nabla \cdot \nabla u \, \text{d}V \]

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\[ \nabla \cdot V_i = 0, \quad \text{i.e.,} \quad \frac{\partial U_i}{\partial r} + \frac{U_i}{r} + \frac{\partial W_i}{\partial z} = 0, \quad i = 1, 2, 3 \quad (4) \]

\[ U_i = -K_{p_i} \frac{\partial P_i}{\partial r}, \quad W_i = -K_{p_i} \frac{\partial P_i}{\partial z}, \quad i = 1, 2, 3 \quad (5) \]

Substituting Eq. 5 into Eq. 4 gives the governing equation for the filtration model,

\[ \nabla^2 P_i = 0, \quad \text{i.e.,} \quad \frac{\partial^2 P_i}{\partial r^2} + \frac{1}{r} \frac{\partial P_i}{\partial r} + \frac{\partial^2 P_i}{\partial z^2} = 0, \quad i = 1, 2, 3 \quad (6) \]

Axisymmetry at \( r = 0 \) and no flux at \( r = \xi \) (periodicity or having reached the leaflet edge) give

\[ U_i = -K_{p_i} \frac{\partial P_i}{\partial r} = 0 \quad \text{at} \quad r = 0 \quad \text{and} \quad r = \xi, \quad i = 1, 2, 3 \quad (7) \]

as the \( r \) boundary conditions. By definition, \( P_a = 1; \ P_v = 0. \) Let \( \Delta R = R_2 - R_1 \) be the average leaky junction width. The \( (\text{linear}) \ z \) boundary conditions are as follows. If there is a leak from \( R_1 < r < R_2 \) at \( z = 0, \)

\[ W_1 = -K_{p_1} \frac{\partial P_1}{\partial z} = L_{p_1} (1 - P_1) \quad \text{at} \quad 0 < r < R_1, \]

\[ R_2 < r < \xi, \quad \text{and} \quad z = 0 \quad (8a) \]

\[ W_1 = -K_{p_1} \frac{\partial P_1}{\partial z} = L_{p_1} (1 - P_1) \quad \text{at} \quad R_1 < r < R_2, \]

\[ \text{and} \quad z = 0 \quad (8b) \]

If there are no leaks at \( z = 0, \)

\[ W_1 = -K_{p_1} \frac{\partial P_1}{\partial z} = L_{p_1} (1 - P_1) \quad \text{at} \quad z = 0 \quad (9) \]

If there is a leak from \( R_1 < r < R_2 \) at \( z = 1, \)

\[ W_2 = -K_{p_1} \frac{\partial P_2}{\partial z} = L_{p_2} P_3 \quad \text{at} \quad 0 < r < R_1, \]

\[ R_2 < r < \xi, \quad \text{and} \quad z = 1 \quad (10a) \]

\[ W_3 = -K_{p_1} \frac{\partial P_3}{\partial z} = L_{p_1} P_3 \quad \text{at} \quad R_1 < r < R_2 \text{ and } z = 1 \quad (10b) \]

And if there are no leaks at \( z = 1, \)

\[ W_3 = -K_{p_1} \frac{\partial P_3}{\partial z} = L_{p_1} P_3 \quad \text{at} \quad z = 1 \quad (11) \]

If there is a leaky cell, \( 0 < r < R_1 \) and \( R_2 < r < \xi \) denote the endothelium with \( \text{EG}_3 \) and normal junctions.

At the interfaces \( z = L_1 \) and \( z = L_1 + L_2 \) between the middle layer and the aortic and ventricular aspects’ intimae, pressure and normal velocity are continuous, giving matching conditions

\[ P_1 = P_2, \ W_1 = W_2, \ i.e., \ K_{p_1} \frac{\partial P_1}{\partial z} = K_{p_2} \frac{\partial P_2}{\partial z} \quad \text{at} \quad z = L_1 \quad (12) \]

One solves the governing equations (Eq. 6), boundary conditions (Eqs. 7–11), and matching conditions (Eqs. 12 and 13) for the pressure either numerically or in terms of Bessel functions. Darcy’s law (Eq. 5) yields the velocities needed in the macromolecular transport model described below.

Macromolecular transport model. Macromolecular transport in heart valves satisfies conservation at constant mass density. The model neglects solute binding to extracellular matrix [weeks (8, 15, 19–21)], valid over the timescales, ~30 min, of the experiments considered. It also neglects cellular uptake and metabolism of tracer: healthy intimal extracellular matrix is cell free, and the endothelial surface receptors are normally already completely bound with unlabeled LDL. Only small numbers of free receptors recycle to the cell surface on the experimental timescale. The conservation equation is

\[ \frac{\partial C_i}{\partial t} + \nabla q_i = 0, \quad i = 1, 2, 3 \quad (14) \]

The constitutive equation for the dimensionless solute flux vector \( q_i = (q_{ix}, q_{iz}) \) has convective and diffusive (effective diffusion coefficient \( D_i \)) contributions in \( r \) and \( z \) defined by

\[ q_{ix} = -D_{xu} \frac{\partial C_i}{\partial r} + \text{Pe}_{ix} C_i \quad q_{iz} = -D_{zu} \frac{\partial C_i}{\partial z} + \text{Pe}_{iz} C_i \quad (15) \]

\[ C_i = \frac{C^*_i}{C^*_0} \quad \tau = \frac{D^*_x}{L^*}, \quad D_i = \frac{D^*_i}{D^*_z} \quad (16a) \]

\[ \text{Pe}_{ix} = \frac{f_i L^*_u}{D^*_x} \quad \text{Pe}_{iz} = \frac{f_i L^*_u}{D^*_z} \quad q_{iu} = \frac{q^*_u}{D^*_z C^*_0 L^*} \quad q_{iz} = \frac{q^*_z}{D^*_z C^*_0 L^*} \quad (16b) \]

\( C^*_i \) is the local dimensional macromolecular concentrations (averaged over all phases, tissue and fluid, present locally) in layer \( i = 1, 2, 3 \). \( C^*_0 \) is the plasma macromolecular concentration, \( \tau \) the nondimensional time, and \( D_i \) effective diffusion coefficient. \( f_i \) is the retardation coefficient, the ratio of solute velocity to water velocity, and \( \gamma_i \) is the volume fraction for macromolecules, the fractional volume available for macromolecules per unit total tissue volume. \( \text{Pe}_{ix} \) and \( \text{Pe}_{iz} \) are the Péclet numbers in \( r \) and \( z \), whose velocities, \( U^* \) and \( W^* \), come from the filtration model.

Because the width of the leaky junction (~20–100 nm; Ref. 7) is comparable to the diameters of macromolecules, e.g., LDL ~23 nm, that traverse it, the macromolecular concentrations at the subendothelial and lumen sides of the leaky junction differ (40, 43). According to Tzeghui et al.’s (37) simple 1D, Cartesian, quasi-steady convection-diffusion model, shown in Fig. 2B, the nondimensional macromolecular flux \( q_i \) through the leaky junction is \((x \) is normal to the endothelial surface):

\[ q_i = -\frac{\partial C_i}{\partial x} + \text{Pe}_{ix} C_i \quad (17a) \]
\( q_i = \frac{f L W_i}{D_i^w}, \quad \alpha_i = \frac{q_i}{D_i^w C_p^w L_i}, \quad C_i = \frac{C_i^w}{C_p^w}, \quad x = \frac{x_i^w}{L_i} \) \hspace{1cm} (17b)

\( L_i \), \( Pe_i \), \( f_i \), and \( D_i^w \) are the endothelial height (or length of the leaky junction), Péclet number, retardation coefficient, and effective diffusion coefficient in the leaky junction, respectively. The solute concentration, \( C_i \), depends on \( x \). The model is Cartesian because the junction thickness is three orders of magnitude smaller than the EC radius. As such, \( W_i^w \) in the leaky junction is uniform (so is \( Pe_i \) well defined), as is the steady-state \( q_i \). Equation 17a directly integrates to relate \( q_i \) and \( C \) at the junction exits, \( C_i \). This gives Eq. 18 at the aortic aspect where \( C_i(x = 0) = 1, C_i(x = 1) = C_{ls} \) and Eq. 19 at the ventricular aspect where \( C_i(x = 0) = C_{ls}, C_i(x = 1) = 1. \)

\[
q_i = Pe_i \frac{\exp(Pe_i) - C_i}{\exp(Pe_i) - 1} \quad (18)
\]

\[
q_i = Pe_i \frac{\exp(-Pe_i) - C_i}{\exp(-Pe_i) - 1} \quad (19)
\]

The flux and concentration in the fluid are continuous at layer interfaces, requiring matching as:

\[
q_{l1} = \frac{C_1}{\gamma_1} = \frac{C_2}{\gamma_2} \quad \text{at} \quad z = L_1 \quad (25)
\]

\[
q_{l2} = \frac{C_2}{\gamma_2} = \frac{C_3}{\gamma_3} \quad \text{at} \quad z = L_1 + L_2 \quad (26)
\]

The apparent concentration discontinuities arise from the concentration variables being defined in a per-unit total volume sense instead of a per-unit fluid volume available to the solute sense.

When the tracer reaches the lumen adjacent to the valve leaflet, the tissue is tracer free:

\[
C_i = 0 \quad \text{at} \quad \tau = 0, \quad i = 1, 2, 3 \quad (27)
\]

The APPENDIX details the numerical methods for solving the filtration and mass transfer problems.

**Constants and parameters.** Table 1 summarizes the baseline values of the constants/parameters in the models. There is far less experimental data and theoretical work on the valvular than the arterial endothelium. The endothelium is one of the largest “organs” in the body (17). As such, and because of the paucity of data, we assume identical endothelial parameters for both aspects, i.e.,

<table>
<thead>
<tr>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( L_i(L_i), ) mm</td>
<td>200</td>
</tr>
<tr>
<td>( L_i, ) ( \mu )m</td>
<td>2</td>
</tr>
<tr>
<td>( L_{ls}, ) ( \mu )m</td>
<td>100, 99.6</td>
</tr>
<tr>
<td>( R_i, ) ( \mu )m</td>
<td>11.7</td>
</tr>
<tr>
<td>( \Delta R_i, ) nm</td>
<td>35</td>
</tr>
<tr>
<td>( \Delta R^*, ) mmHg</td>
<td>50</td>
</tr>
<tr>
<td>( L_p^w, L_{p,v}^w, ) cm( ^3 )-s(^{-1} )-mmHg(^{-1} )</td>
<td>( 1.18 \times 10^{-10} )</td>
</tr>
<tr>
<td>( L_p^w, L_{p,v}^w, ) cm( ^3 )-s(^{-1} )-mmHg(^{-1} )</td>
<td>( 7.09 \times 10^{-7} )</td>
</tr>
<tr>
<td>( K_{p,v}^w(25 \times), ) cm(^2 )</td>
<td>( 1.10 \times 10^{-12} )</td>
</tr>
<tr>
<td>( K_{p,v}^w(25 \times), ) cm(^2 )</td>
<td>( 2.28 \times 10^{-16} )</td>
</tr>
<tr>
<td>( \gamma_1(\gamma_3) )</td>
<td>0.60 (LDL)</td>
</tr>
<tr>
<td>( \gamma_2 )</td>
<td>0.92 (HRP)</td>
</tr>
<tr>
<td>( f_i )</td>
<td>1.0</td>
</tr>
<tr>
<td>( f_i(f_v) )</td>
<td>0.84 (LDL)</td>
</tr>
<tr>
<td>( f_v )</td>
<td>0.56 (LDL)</td>
</tr>
<tr>
<td>( 4.09 \times 10^{-10} ) (HRP)</td>
<td>44</td>
</tr>
<tr>
<td>( 5.93 \times 10^{-9} ) (LDL)</td>
<td>2.27 \times 10^{-8} (HRP)</td>
</tr>
<tr>
<td>( 1.63 \times 10^{-8} ) (LDL)</td>
<td>7.2 \times 10^{-3}</td>
</tr>
<tr>
<td>LDL, low-density lipoprotein; HRP, horseradish peroxidase. a, ( L ) is average value from Ref. 34, and ( L_3 = L - L_4 - L_5; ) b, ( L_{p,v}^w, L_{p,v}^w = (\Delta R^*/12\mu L_i) (45); ) c, fitted results; d, estimated from ( D^v_{23HRP}/D^v_{23LDL} \approx r_{LDL}/r_{HRP} ) and ( \gamma_{23HRP}/ \gamma_{23LDL} \approx \gamma_{23LDL}/\gamma_{23LDL}. )</td>
<td></td>
</tr>
</tbody>
</table>
Moreover, we adopt values for the endothelial hydraulic conductivities $L_{p_a}^*$ and $L_{p_v}^*$, as well as the parameters of the leaky junction, $L_i$, $f_i$, and $D_{i}^*$, from those of the arterial endothelium. We also assume that the valvular intimae at the aortic and ventricular aspects also have identical properties and structures, i.e.,

$$\gamma_1 = \gamma_2, \quad K_{p_a}^* = K_{p_v}^*, \quad D_{p_a}^* = D_{p_v}^*, \quad f_1 = f_3, \quad L_1 = L_3,$$  \hspace{1cm} (29)

Huang et al. (16) used a heterogeneous fiber matrix theory (18) to calculate the permeability and macromolecular diffusion coefficient of the aortic intima based on the typical fiber thickness and spacing of its extracellular matrix. They (and we) measure the average fiber radius and spacing from J. Frank’s group’s ultrarapid freezing/rotary shadow etchings for the aortic intima [here (11)] and the valve intima [here (22)]. The etchings show both thin (proteoglycans) and thicker (collagen) fibers. One calculates (see details in Ref. 16) an average fiber radius for the proteoglycans and assumes a regular hexagonal array of these average-radius fibers with the given fiber spacing (here 30–40 nm). One then calculates the total fiber number per unit volume and sets fractional void volume $e$ equal to $1 - \text{total fiber volume per unit total volume}$. Reference 16 also estimates $e$ due to the collagen. Curry (9) sets $\gamma = \exp[(1 + e)(a/r_1 + 1)^2]$, where $a$ and $r_1$ are the radii of solute and fiber, respectively, when there is a single fiber present. Since both collagen and proteoglycan are present, we set $\gamma$ equal to the product of two such factors, one for each fiber type. By varying the fiber spacing from 30 to 40 nm and the geometric parameters describing the proteoglycan structure over the ranges defined in Ref. 16, we find that $\gamma_1$ varies from 0.49 to 0.63; we work with $\gamma_1 = 0.6$. In Fig. 11, we demonstrate that using 0.5 (curve 2b) leads to a negligible change in the results, compared with curve 2. Because of the almost identical characteristic sizes that one measures from ultrarapid freezing/rotary shadow etchings for the valvular intima (22, 46) and the aortic intima (11, 16), this fiber matrix theory predicts that $K_{p_a}^*$, $K_{p_v}^*$, and $D_{p_a}^*$, $D_{p_v}^*$ are nearly equal to the corresponding aortic intima parameters. Part I (46) estimates the thickness, $L_i(L_3)$, of the intima. Because of the paucity of the data, unless otherwise indicated we do not distinguish between the rat (16, 46) and squirrel monkey [Tompkins et al. (34)] aortic valve endothelial and intimal parameters. Reference 34 measured the volume fraction, $\gamma_2$, in vitro. Although, strictly speaking, Ref. 34 only measured the volume fraction of the whole valve leaflet, this value is very close to $\gamma_2$, because the valvular intima comprises a negligible fraction of the whole valvular leaflet. The retardation coefficient, $f_2$, relates to the volume fraction via (9)

$$f_2 = 1 - (1 - \gamma_2/\gamma_{v2})^2,$$  \hspace{1cm} (30)

where $\gamma_{v2}$ is the fractional volume, 0.5 (32), available for water in the middle layer. If one neglects the very small pressure difference between the ventricle and aorta during systole, one can roughly estimate $\Delta P^w$ from the aorta’s diastolic blood pressure multiplied by the diastole fraction of the cardiac cycle. We have devised a fitting scheme, based on one of the transvalvular LDL concentration profiles in Fig. 1, for the remaining three valvular transport parameters, $k_a(k_v)$, $K_{p_a}^*$, and $D_{v}^*$. According to our 2D model’s Ansatz, once fitted, these parameters are fixed, at least for any aortic valve from the same species of (normal) animal. Our working hypothesis is that the observed variations between the transvalvular LDL concentration profiles in Fig. 1 result simply from the number and location of leaky cells in the valvular endothelium and the location, relative to such leaks, from which the tissue sections were taken. If, for a particular set of serial sections, there happened to have been no leaks in the endothelia of either aspect of that aortic valve, the corresponding transvalvular LDL concentration profile would be the lowest possible. This follows because, other than through leaky junctions, LDL has difficulty traversing the normal endothelium. The corresponding profile should have a slightly higher concentration at $z = 1$ than at $z = 0$ because of the small overall convection toward the ventricular aspect that is present. Profile 9, the lowest profile, in Fig. 1 is the most likely representative of this case. Conveniently, in the absence of leaks in either aspect, the filtration and transport models reduce to 1D, with only a single spatial variable $z$. All dependent variables become independent of $r$, i.e., $\partial W/\partial r = 0$, and Eqs. 4, 6, and 14 simplify dramatically to

$$\frac{dW_i}{dz} = 0,$$  \hspace{1cm} (31)

$$\frac{dP_i}{dz} = 0,$$  \hspace{1cm} (32)

$$\frac{\partial C_i}{\partial \tau} + \frac{\partial q_{ie}}{\partial z} = 0, \quad i = 1, 2, 3,$$  \hspace{1cm} (33)

with all the (no leak) boundary and matching conditions in $z$ direction the same as the 2D model except for setting $\partial \tau/\partial r = 0$. It is obvious that $W_i$ and $P_i$ are uniform and linear functions of $z$, respectively, and lend themselves to easy analytical solutions with the corresponding boundary and matching conditions. With the substitution of this velocity into the convection-diffusion equation, Eq. 33, the concentration distribution lends itself to numerical solution by the standard Crank-Nicolson implicit finite difference method (30). (The matching conditions make it difficult to ascertain self-adjointness, which would be necessary for a direct analytic solution in terms of separation of variables, and the Laplace transform solution has far too many terms to be enlightening.) Thus one can obtain the remaining three valvular transport parameters, $k_a(k_v)$, $K_{p_v}^*$, and $D_{v}^*$, by fitting the 1D convection-diffusion model, Eqs. 31–33, to concentration profile 9 in Fig. 1. In this fitting, the search for the least square error between the theoretical and experimental values is a complicated global minimization process. There are no explicit functional expressions to describe the relationship between the square error and the values of the three independent variables, and the square error may have a large number of local minima. We choose Bremermann’s method of unconstrained global optimization (2, 3) to find optimized values of $k_a(k_v)$, $K_{p_v}^*$, and $D_{v}^*$ that minimize the square error. This method is based on the chemotactic behavior of bacteria and efficiently and rapidly finds very good maxima or minima of a real-valued function of many variables, even when the function has many local maxima and/or minima.
**RESULTS AND DISCUSSION**

Fitted results of valvar transport parameters. The smooth curve in Fig. 3 is the fit of the no-leak model case to the data (discrete points) from Fig. 1’s profile 9 with Bremermann’s method (2, 3). The three parameters control different aspects of the fit. \(k_v\) (\(k_v\)) control the curve’s overall height, \(K_p\), the asymmetry toward the ventricular aspect, and \(D_p\) its flatness. The fitted mass transfer coefficients, \(k_v\) (\(k_v\)), assumed equal, for LDL across the endothelia of both aspects of the squirrel monkey’s aortic valve leaflet, are \(1.63 \times 10^{-3}\) cm/s. This is consistent with the measured value (LDL), \(1.9 \pm 0.8 \times 10^{-8}\) cm/s, of rabbit aortic endothelium (36), which supports our assumption that the valvar endothelia share similar transport properties with the arterial endothelium. The fitted effective diffusion coefficient for LDL is \(D_p = 5.93 \times 10^{-9}\) cm²/s, and the permeability \(K_p = 2.28 \times 10^{-16}\) cm² in the middle layer. This value of \(D_p\) is very close to the average of Tompkins et al.’s values for the diffusivity of the lumped middle layer. Both parameters are much smaller than those theoretically calculated from fiber matrix theory for the valve intima, \(D_I = 1.02 \times 10^{-7}\) cm²/s ( LDL) and \(K_p = 1.1 \times 10^{-12}\) cm² (16). This is consistent with the tissue structure of the aortic valve’s middle layer [with its 3 layers, the lamina fibrosa, lamina spongiosa, and ventricularis, lumped together into a single, uniform effective medium (10, 42)] being much denser than that of its intimae. That the volume fraction for LDL in the middle layer, \(K_p\) of its intimae. That the volume fraction for LDL in the middle layer’s matrix may be slightly denser (on average over its denser lamina fibrosa and ventricularis and looser lamina spongiosa) than the aortic media’s. LDL space in the valve middle layer is in the range \(10\), \(10\) cm/s, estimated in the arterial media from a 1D model including both convection and diffusion. Paradoxically, the middle layer hydraulic conductivity, \(L_p = K_p/(\mu L_2) = 0.42 \times 10^{-8}\) cm·s⁻¹·mmHg⁻¹, appears smaller than the measured hydraulic conductivity, \(L_p\) of rat (4.76 \(\times 10^{-8}\) cm·s⁻¹·mmHg⁻¹; Ref. 27) or rabbit (5.36 \(\times 10^{-8}\) cm·s⁻¹·mmHg⁻¹; Ref. 33) aorta with denuded endothelium, i.e., media plus a very small internal elastic layer (IEL) correction. These numbers require discussion. Slight overestimate of \(L_p\) due to media hydration (27) or due to erroneously setting the valve’s and aorta’s endothelial \(L_p\) equal, despite known differences between the two [e.g., their ECs align differently with flow (41)] is unlikely to be responsible. That \(L_p = L_p = \sim 3 \times 10^{-8}\) cm·s·mmHg⁻¹ \(> L_p\), where \(L_p\) is the \(L_p\) of intact aorta, negates the former, while small errors in the latter are likely negligible since the leaflet’s middle layer resistance dwarfs its endothelial resistance. This \(K_p\), does not appear to be in error: one can try using the aortic medial \(K_p\) value instead of \(K_p\), in the calculation of LDL profiles corresponding to Tompkins et al.’s measurements below. The magnitude of transvalvular convection becomes so large that it sweeps all calculated profiles discussed below significantly toward the ventricular aspect, where they become far too high. The disparity in \(K_p\) and in LDL diffusivities does not contradict Weind et al.’s finding that the effective oxygen diffusivity in the aorta, femoral vein, and valve leaflet are all in the same range. Their measured \(D_p\) values derive from measurements of oxygen arriving at a sensor at one end of a porcine leaflet interpreted as solely arriving due to diffusion, i.e., using models that do not include convection. The fit diffusivity then actually represents the combined effects of diffusion and convection, and its value being similar in the arteries and the leaflet is consistent with one parameter being higher and the other lower in the leaflet, by roughly the same factor.

The paradox may simply be due to species dependence of the precise parameter values. Alternatively, it is possible that both the \(D_p\) and \(K_p\) ratios are indeed approximately correct. The valve middle layer’s matrix may be slightly denser (on average over its denser lamina fibrosa and ventricularis and looser lamina spongiosa) than the aortic media’s. LDL space measurements in these tissues are not sufficiently sharp to address this, but it appears plausible. Tedgui and Lever (32) find that the aortic media’s albumin space is 0.08, whereas others (5, 12, 35) give values of 0.09–0.17 for it. Tompkins et al. (34) find that the LDL space in the valve middle layer is in the range 0.03–0.16. Despite its higher density, \(D_m\) of the aortic media can still be smaller than \(D_p\) of the valvar middle layer because the aortic media is composed of repeated layers of elastic tissue, separated by smooth muscle cells (SMCs) and extracellular matrix. As such, a large LDL particle diffusing normal to the elastic sheets likely needs to find each elastic sheet’s fenestrae before it can traverse the sheet. Moreover, these particles likely cannot diffuse through SMC, but rather must diffuse around them. As such, the diffusion path through the aortic media may be significantly longer than the medial thickness, thereby resulting in an effective diffusivity that is smaller than that in the elastic-free and nearly cell-free valvar

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**Fig. 3.** Best fit (curve) of the no-leak model case to Fig. 1, profile 9’s data (points) for LDL concentration as a function of depth into the valve using Bremermann’s method (2, 3). C, nondimensional LDL concentration; \(z\), nondimensional distance normal to the endothelium. Asterisks near \(z = 0\) and 1 indicate the segments of the curves in the layers of intimae, which look like points because of the thinness of the intimae.
middle layer, despite the former having the slightly higher LDL space. All of the calculations below employ the parameter values fit above.

**Pressure and velocity distribution.** Figure 4 shows the detailed three-dimensional (3D) pressure distribution near the leaky junction for \( r^* R_1 \leq 0-15 \) and \( z \leq 0-0.22 \) near the intima at the aortic aspect for the case of one leak at the aortic aspect and no leaks at the ventricular aspect. Because the leaky junction has a much larger hydraulic conductivity than the normal junction, the pressure drop across the leaky junction is much smaller than across the normal junctions and therefore the absolute pressure inside the leaflet’s aortic aspect is highest at the leaky site. This value decays to a plateau in the radial direction (parallel to the endothelium), where the effect of the leaky junction vanishes. This radial pressure gradient drives a strong radial convection that rapidly advects tracer, yielding the observed rapidly growing tracer spots. The pressure decreases monotonically in the \( z \)-direction normal to the endothelium toward the ventricular aspect. Figure 4 illustrates that the pressure in the intima has only a slight, nearly linear \( z \) dependence far from the leak. This is the direct result of the scale separation there, i.e., that \( L_z/\xi = O(10^{-4}) \) which leads to a boundary layer-type result for Laplace’s equation in the intima that the pressure is linear in \( z \) to leading (first) order in \( L_1 \). (This scaling is not valid near the leak where variables change in the \( r \) direction over scales, \( \sim 20-30 \) nm, comparable to the junction thickness and no longer large compared with \( L_1 \).) In contrast, the pressure varies appreciably in \( z \) in the thick, high-resistance middle layer, where \( L_z/\xi = O(1) \) and reflects the overall water flow through the leaflet.

Figure 5 presents the 2D pressure profiles in the valve intima at \( z = 0 \) and 1 as a function of radial distance from the center of the leaky cell for various cases of numbers of leaks in the two endothelia, including the no-leak case, the one aortic leak case, and the one ventricular leak case.

Figure 6 plots the corresponding radial velocity profiles (viz., Darcy’s law, Eq. 5). Numerical calculations shows that these curves only depend on the leak situation at the nearby aspect; a change in the leak number at the faraway aspect yields changes in the given curves that are far too small to be seen in the figures. For example, curve 1 in Fig. 5 can represent the local pressure distribution at \( z = 0 \) not only for one leak at the aortic and zero leaks at the ventricular aspect, but also for one leak at each aspect. As Fig. 5 illustrates, the filtration model is symmetric about \( P = 0.5 \) and \( z = 0.5 \), since a simple substitution of \( z \) and \( P_1 \) with \( 1-z \) and \( 1-P_1 \) in the model for the case of either one or zero leaks on both aspects leaves the model unchanged. The same substitution for the case of one leak at one aspect and zero leaks at the other simply switches the location of the leak to the other aspect. Curves 3 and 4 in Fig. 5 are straight lines parallel to the \( x \)-axis, indicating an

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**Fig. 4.** Three-dimensional pressure distribution near the leaky junction in the vicinity of the intima at the aortic aspect for the case of 1 leak at the aortic aspect and no leaks at the ventricular aspect. The axes are nondimensional pressure (\( P \)), nondimensional normal distance (\( z \)), and radial distance (\( r^* \)) divided by radius of the endothelial cell (\( R_1 \)).

**Fig. 5.** Nondimensional pressure (\( P \)) as a function of radial distance (\( r^* \)) divided by radius of the endothelial cell (\( R_1 \)) in the subendothelial intima at fixed \( z \). Curve 1: \( z = 0 \), 1 aortic aspect leak, 0 ventricular aspect leaks; curve 2: \( z = 1 \), 0 aortic leaks, 1 ventricular leak; curve 3: \( z = 0 \), no leaks at either aspect; curve 4: \( z = 1 \), no leaks at either aspect.

**Fig. 6.** Radial velocity (\( U^* \)) as a function of radial distance (\( r^* \)) normalized by the radius of an endothelial cell (\( R_1 \)) in the subendothelial intima at both aspects, dashed line and dotted line corresponding to curves 1 and 2 in Fig. 5, respectively. The 2 curves, reflections of each other about \( U^* = 0 \), show their largest absolute velocities at the edge of the leaky cell at \( r^* R_1 = 1 \), decaying to almost zero far from the leak.
r-independent pressure and thus that there is no radial velocity or convective transport. This serves as a check that the 2D calculation reduces under these conditions to the 1D profile that was invoked in the parameter fit above. The zenith and nadir of pressures in that was invoked in the parameter fit above. The zenith and calculation reduces under these conditions to the 1D profile and thus that there is no radial velocity

A leaky junction at the aortic aspect acts like a fluid source because the pressure has a local maximum there and as a fluid sink at the ventricular aspect, where it has a local minimum. The signs of the velocities in Fig. 6 reflect this. The radial pressure gradient in curves 1 and 2 in Fig. 5 are at the border of the leaky cell, $r^* / R_1 = 1$, and correspond to the maximum/minimum of the corresponding radial velocity curves in Fig. 6. A leaky junction at the aortic aspect acts like a fluid source because the pressure has a local maximum there and as a fluid sink at the ventricular aspect, where it has a local minimum. The signs of the velocities in Fig. 6 reflect this. The radial pressure gradient in curves 1 and 2 in Fig. 5, i.e., the corresponding radial velocity in Fig. 6, is large within a radius of $\sim 6-8 R_1$, with its largest (discontinuous) value, $\sim 10^{-2}$ cm/s, at $r^* / R_1 = 1$, and decays to a tiny amount, $\sim 10^{-7}$ cm/s, at $r^* / R_1 = \sim 15$. Thus the effect of leaky junction on the radial convection in the intima acts at most within a circle of this radius.

Solution of mass transfer problem. Figure 7 is the LDL concentration distribution in the vicinity of the leaky cell near the aortic aspect ($r^* / R_1 \leq 0.15, z \leq 0-0.02$) 30 min after exposure of an initially tracer-free leaflet to a unit step change in lumen tracer concentration at both leaflet aspects. The leaflet has one leak at the aortic aspect and no leaks at the ventricular aspect. Because of the enhanced width of the leaky junction, macromolecular transport through the leaky junction is much larger than through the normal junctions that, in view of Tompkins et al. (34), is not zero. Thus there is a tracer concentration maximum at $r^* / R_1 = 1$ and $z = 0$. For any fixed normal distance $z$ the concentration is the highest at $r^* / R_1 = 1$ and decays very rapidly in the radial direction in the intima. This rapid decay is due not only to the large difference in the rate at which LDL crosses the endothelium between the leak and the nonleak parts of the endothelium but also to the dilution of the LDL as it is advected radially in the intima by water crossing the endothelium everywhere. Note also that the same separation of scales that led us to conclude that the pressure was linear in $z$ in the intima to leading order in the small parameter $L_1 / \xi$ far from the leak also implies that the C is independent of $z$ in the intima to leading order in $L_1 / \xi$ there. The numerics illustrate this. The intima C clearly depends on $r$ and is largest near the leak.

In the $z$-direction there is a sharp discontinuity in LDL concentration at the intima-middle layer interfaces because of the mismatch in the void space between these regions and the definition of concentration on a per-unit total volume (and not per-unit void) basis that we use throughout. The matching conditions, Eqs. 25 and 26, express this and lead to step changes in the concentration distribution according to the ratio $\gamma_1 / \gamma_2$. For any fixed radial position $r$, the concentration is the highest at $z = 0$ and 1 and decays to its lowest point in the middle layer, as in earlier (10, 42) diffusion-only oxygen transport studies. This minimum is near, but generally not exactly (because of the symmetry breaking directionality of the transvalvular convection), $z = 0.5$ for the cases of zero or one leak at both aspects, near $z = 1$ for the case of only one leak at the aortic aspect and no leaks at the ventricular aspect, and near 0 for the opposite case. The influence of the leaky junction on the concentration distribution appears to be limited to within $\leq 10-11 R_1$ in the radial direction. At further distances the concentration distribution decreases monotonically and is almost identical to the case of no leaks at both aspects. That is, far from the leak in the radial direction the leaflet does not know that there is a leak.

Figure 8 plots the LDL concentration at $t = 30$ min as a function of radial distance at fixed $z = 0$ and 1 for the same four leak arrangements handled in Fig. 5. Again, as in Fig. 5, these curves depend strongly only on the leak situation at the proximal aspect and a change in the leak status of the far aspect changes the curves imperceptibly. These curves illustrate the effect of the leaky junction on the mass transport 30 min after the step change in lumen LDL concentration. As discussed above, a leak in the aortic aspect causes a radial flow, and consequently mass transport, in the intima away from the leak. Curve 1, at $z = 0$ for one leak in the aortic aspect, illustrates

![Fig. 7. LDL concentration distribution near a leaky junction at the aortic aspect in the vicinity of the aortic aspect’s intima. The 3 axes are nondimensional concentration (C), nondimensional normal distance (z), and radial distance ($r^*$) divided by radius of the endothelial cell (R1).](http://ajpheart.physiology.org/)
this case. In contrast, curve 3 represents a leak in the ventricular aspect, where convection sweeps tracer toward and exits through the leaky junction into the lumen. As expected, curves 1 and 3 have their maxima at the leaky site, $r^0/R_1 = 1$. Since the lumen LDL concentration is higher than that of the tissue, convection at the aortic aspect sweeps tracer in the same direction as diffusive transport, whereas convection at the ventricular aspect sweeps it in the direction opposite to diffusion. Therefore, curve 1 is higher than curve 3 near $r^0/R_1 = 1$. Both curves exceed the leak-free curves 2 and 4 because of the enhanced transport through the leak. Curve 1 (3) decays to curve 4 (2) at fixed $z = 0$ (1) at large $r^0/R_1$ for the case of no leaks at either aspect, suggesting that the influence of the leaky junction on the tracer’s concentration profile at 30 min is confined to $\sim 10$–$11R_1$. Curves 2 and 4 are straight lines parallel to the x-axis. Curve 2 slightly exceeds curve 4, because of the small normal convection. Similarly, in Fig. 3 the value at $z = 1$ is a little higher than that at $z = 0$.

**Growth of macromolecular leakage spots.** Experiments described in Part I (46) measured the radii of HRP leakage spots as a function of HRP circulation time in the rat valve leaflet. The maximum circulation time was 4 min since for longer times HRP, with a diameter of only 6 nm (16), penetrates the normal junctions. This results in a difficulty in distinguishing leaky spots from background. As in similar experiments in the aorta, one does not know a priori the critical concentration $C_c$ of HRP that is detectable as a dark spot. This threshold depends on the complicated conditions of the biochemical staining reaction and photographic exposure. Another issue is that it takes a finite time for HRP to reach the aortic valve from the point of HRP injection, the femoral vein. Consequently, there is an unknown time lag, $t_0$, between the theoretical and the experimental initial times that is not directly available from the experimental data. For the aorta, a trial and error variation of the theoretical delay matched the data when $t_0$ was $\sim 25$ s (16). This procedure calculated the intimal HRP concentration as a function of $r/R_1$, for different values of the time $t - t_0$ over which we have solved the initial value problem corresponding to the time since the tracer reached the leaflet. For each of a series of values for $t_0$, we determined $C_c$ as the HRP concentration at $t$ that corresponded to the experimental radius of the experimental time $t = 30$ s. We then assume that $C_c$ is the same for all four experimental circulation times and therefore simply read off the values of $r/R_1$ at which the horizontal line at $C_c$ intersects to intimal profiles from the $t = 60$, 120-, and 240-s curves. We then pick the value of $0 < t_0 < 30$ s giving the curve that best fits the data. Because of the proximity of the aortic valve to the aorta, we expect this procedure applied here to yield essentially the same value.

Whereas the physical basis of the theory developed above rests on data in the rat from Part I, we have assumed that these qualitative features apply in the squirrel monkey as well and have fit three of the parameters from the monkey data. Although our main goal in this paper is to explain the monkey data, we would like to test whether, under the assumption that the monkey and rat parameters are similar, the above theory can qualitatively explain Part I’s HRP spot growth data as well. Other than $t_0$ there are no parameters to fit, and $t_0$ will only be reasonable if it is close to the aorta’s value. Figure 9 plots the evolution of the intimal concentration as a function of radial position in the aortic aspect’s intima at $z = 0$ from the theory above. The time-dependent concentration curves correspond, from lowest to highest, to HRP circulation times of 30, 60, 120, and 240 s minus the time lag $t_0$, which we found here to be 25 s as in the aorta. Since the fiber size and spacing, as measured from numerous (both published and unpublished) ultrarapid freezing/rotary shadow etchings in the valve intima (22) and the aortic intima (11), appear to be very similar, the fiber matrix theory that calculates values for $\gamma_1$ and $D_1^\star$ for HRP is the same for these two intimae; we thus use the aortic values (16, 44). In Refs. 16 and 44 we showed that the aortic intima values for these parameters exceeded the medial values by one to two orders of magnitude, a qualitative, not quantitative, difference. Moreover, plots of parameter values as functions of the precise fiber spacing or thickness showed a weak variation. As such, the results calculated here are robust with respect to variations of a factor of two in fiber parameters. We estimate $\gamma_2$ for HRP by simply assuming the same ratio $\gamma_1/\gamma_2$ for LDL as for HRP. Motivated by the Stokes-Einstein equation (see Ref. 9), we estimate $D_2^\star$ for HRP by using the simple relationship of $D_2^\star(BP)/D_2^\star(LDL) \approx r_{LDL}/r_{HRP}$, where $r_{LDL}$ and $r_{HRP}$ are the radii of LDL and HRP molecules, respectively, even though here $D_2^\star$ is the effective diffusion coefficient instead of the molecular diffusivity. Our calculations indicate that, as long as $\gamma_2/\gamma_1$ is small enough to cause the intima flow near the leak to be essentially radial, the early-time transport behavior near the leaky site is not strongly dependent on the precise middle layer transport parameter, $\gamma_2$ and/or $D_2^\star$, values. Convection and diffusion in the intima are its main determinants.

The concentration profiles in Fig. 9 yield a critical value of HRP concentration, $C_c = 0.014$, corresponding to $t_0$ that gives excellent agreement between the theoretically predicted values and experimental results (see the solid line in Fig. 10). In fact, the steepness of the curves in Fig. 9 near $C_c$ makes it clear that the result is insensitive to the precise value of the critical value.

![Fig. 9. Time evolution of horseradish peroxidase (HRP) concentration (C) in the valve’s subendothelial intima at fixed $z = 0$. The time-dependent concentration curves, from lowest to highest, correspond to HRP circulation times ($t$) of 30, 60, 120, and 240 s after injection, respectively, with an estimated time lag, $t_0$, of 25 s. The radial distance $r^0$ is normalized by the radius of an endothelial cell ($R_1$). The straight line represents the threshold HRP concentration $C_c = 0.014$.](http://ajpheart.physiology.org/www.ajpheart.org)
Interestingly, these results show that a dominant radial flow advecting tracer in the intima near the leak comes about as a consequence of the porosity, and hence permeability, mismatch between the intima and middle layers, even in the absence of an IEL separating these layers. In the aorta, we had speculated that the IEL was the lead actor in directing the radial intimal flow. Our valve calculation suggests it more likely plays a subsidiary role, likely accounting only for the ~25% growth difference of short-time HRP spots between the valve and the aorta.

**Explanation of Tompkins et al.’s quantitative study.** Our theory now explains Tompkins et al.’s (34) experimental LDL concentration profiles across the aortic valve of squirrel monkey (Fig. 1) and their variability with a single set of fixed parameters. Before comparing with the model’s predictions, there remain two factors, not recorded in the experimental results, that need to be specified, the number and location of the leaky cells in the leaflet and the position of the tissue sections relative to the leaks’ positions. These issues were not relevant for earlier uniformly permeable endothelium models.

The frequency of the leaky cells in the rat’s arterial endothelium’s most susceptible regions is ~1 cell in 2,000–6,000 (45). If the valvular endothelium has the same frequency, there should be roughly 0–2 leaky cells at any time on each valve aspect and, from Part I, we find 0–3 HRP spots per leaflet. For the cases of 0–1 leaky cells on either aspect’s endothelium, we easily solve the model with the above method and the corresponding boundary conditions, Eqs. 8–13 and 20–26.

Each of Fig. 1’s profiles is the average of *n* (given in the figure) thin serial sections. Tompkins et al. (34) magnified each section to fit a standard grid and counted the grains in the autoradiographic emulsions between grid lines. Our model represents each section as a cross section of an idealized cylindrical domain, whose radius ξ (see *Model description*) depends on the number/location of leaks per leaflet, sliced parallel to its z-axis that projects to a chord of the circle. The perpendicular distance, *r*<sub>s</sub>, from the circle’s center specifies the slice/chord’s location, *L*<sub>s</sub> := (2(ξ<sup>2</sup> + *r*<sub>s</sub><sup>2</sup>)<sup>1/2</sup>) denotes its length, and the width listed in Fig. 1 gives the tissue thickness or its width in the z-direction. For given numbers of leaks on each aspect, we calculate the concentration *C*(*r*, *z*), *i* = 1, 2, 3, at the experimental time *t* = 30 min, and then for each *z* average this concentration over the chord, with length *L*<sub>s</sub> at *r*<sub>s</sub>. Since Fig. 1 (34) predates the concept of localized endothelial leaks, it reports neither *r*<sub>s</sub> nor *L*<sub>s</sub> (<br>http://ajpheart.physiology.org/ Downloaded from AJP-Heart Cir Physiol  • VOL 292 • JUNE 2007 • www.ajpheart.org) via leak number/position) to fit its curves. Below, even by considering only *r*<sub>s</sub> = 0, i.e., the tissue sections close to the leaky site, and just varying *L*<sub>s</sub> we can accomplish this task. One can a fortiori accomplish it by varying both, which suggests multiple possible explanations for each curve.

In principle, since the data are discrete concentrations, we should also average over the Δ*z* corresponding to the grid spacing, but we find that this extra step typically makes little difference. Thus we present the theoretical curves as continuous in Fig. 11, which reproduces Fig. 1’s data (points) at the same scale along with our corresponding theoretical curves. Each theoretical curve has a discontinuous jump at each intima-middle layer boundary to a much higher concentration intima segment of width ~1(1/50)Δ*z* near *z* = 0 and *z* = 1. These jumps again result from the matching conditions, Eqs. 25 and 26, and the discontinuity in LDL space between the intima, γ<sub>1</sub>...
(γ3), and the middle layer, γ2. We list the intima concentration values in the legend to Fig. 11, but not in the figure itself. They serve to very slightly raise the integrated value of the curve at the first and last Δz to compare with the data. Finally, we scale each prediction by the z-scale given in Fig. 11 for that curve.

We begin with profile 3. Like curve 9 used to fit the parameters, its shape has only slight convective skewing toward and a slightly higher concentration value at the ventricular than at the aortic aspect. We postulate that it is also the zero-leak case and therefore has the same theoretical curve with the lowest possible magnitude.

Profiles 2, 5, 10, and 11 have very similar shapes, with the concentration higher near z = 0 than near z = 1, but different magnitudes. We test whether one aortic aspect leak and no ventricular aspect leaks within 15R1 from the section in question (so it does not interact) can explain all four profiles, with their differing magnitudes due to different La, from different leak positions relative to the leaflet center for r = 0. Generally, the closer the leak is to the edge the shorter La (this ignores any local nonaxisymmetry—see formulation) and the higher the curve’s magnitude. The curves (curves 5 and 11’s leak near, curves 2 and 10’s increasingly further from the leaflet center) fit the profiles very well. The legend of Fig. 11 gives all curves’ La values. Curve 10 is far higher in magnitude than the other three curves, and therefore its La (and ξ) is smaller, either because the aortic aspect leak is fairly close to the leaflet edge or because there is more than one aortic aspect leak. A smaller ξ means entering tracer spreads over a smaller area, giving a higher C near the leak.

Before proceeding to the other curves, we test the sensitivity of our calculation to the assumptions regarding Lpna (or Lpnv) and kα (or kν) in Eqs. 8a, 10a, 11a, 20b, and 22b, for 0 < r < R1 and to our choice of γ1 = 0.6. We recalculate profile 2 for zero endothelial parameters for 0 < r < R1 and plot it in curve 2a. We also redo the calculation for the second experimental profile with γ1 = 0.5 (curve 2b). The former perceptibly affected C only for 0 < r < R1, left the spot size curves unchanged, and led to a maximum difference compared with curve 2 of 4.9% in the intima (~2% of a Δz) and 0.7% in the media. When averaging for the end point Δz, these differences are completely negligible. The different γ1 also yielded a completely negligible change to the result.

Profile 7 has a very different shape: a higher concentration near z = 1 than near z = 0. It likely represents one ventricular aspect and no aortic aspect leaks. Curve 7 plots this case, with La chosen to match the data’s magnitude. The curve fits quite well except for the last data point, which is far higher than both the theoretical curve and all of profile 7’s other data points. We
speculate that this point’s anomalous magnitude comes about because it is probably very near the intima. Because of its far sparser structure, the intima’s per-unit total volume tracer concentration is much higher than that in the middle layer. If the intima was thickened there, its high concentration would weigh more heavily in an average of the theoretical profile over that data point’s \( \Delta z \). Since profile 7’s leaflet thickness is 124 \( \mu m \) and it has 18 data points, \( \Delta z \approx 7 \) \( \mu m \). The average intima and middle layer nondimensional concentrations there are 0.074 and 0.019. Thus a local intima thickness of \( \sim 1.4 \) \( \mu m \) would match the theory to the experimental value.

We now turn to profiles 4, 6, and 8. These profiles have higher \( z = 0 \) and \( z = 1 \) concentrations than profile 9, and they are more symmetric in \( z \) than the previous profiles. These therefore likely all represent the case of one leak at each aspect, whose projections into the endothelial plane are close enough to interact. As before, the profiles’ different magnitudes likely derive from their different \( \xi \). Since a ventricular aspect leak has a smaller footprint than an aortic aspect leak, if both leaks are \( a = 0 \) to preserve model axisymmetry, the theoretical curve will always be higher at \( z = 0 \) than at \( z = 1 \). Since the data for these curves are more symmetric in \( z \), the two leaks are likely close, but not aligned (i.e., not both at \( r = 0 \)), where the tissue sections have been cut closer to the leak at \( z = 1 \). A proper calculation would be fully 3D. Since a leak affects \( C(r, z, t) \) only locally in \( z \) and does not change \( C(r, z, t) \) of the opposing aspect’s intima appreciably (cf. Figs. 5, 6), one can approximate \( C(r, z, t) \) due to misaligned leaks by a careful superposition of the results of two axisymmetric calculations, each with a single leak at one aspect and no leaks at the other, taking care not to count the macromolecular permeability of the normal junctions twice. Curves 4, 6, and 8 are the results for this superposition for leaks \( \sim 28 \) \( \mu m \) apart, with the section taken through the leak at \( z = 1 \). \( L_s \) values are 316, 158, and 47 \( \mu m \), respectively, for these curves. Note that profile 8’s data look unusually flat and the model results have somewhat more curvature.

Profile 1 presents our model with its most difficult challenge. Although, like profile 7, its concentration at \( z = 1 \) is higher than at \( z = 0 \), its shape differs from and its magnitude is much higher than any of the other data sets. In particular, the rightmost 4–5 data points have concentrations that approach the volume fraction \( \gamma_z = 0.17 \) of the middle layer times the nondimensional lumen LDL concentration of 1. A \( K_{p,v}^* \) value much larger than what we have used would sweep tracer toward \( z = 1 \) and lead to a higher \( C \) there, but nothing approaching 0.17. One can only rationalize such high LDL concentrations if the endothelium in this leaflet section had been denuded. Curve 1 performs this calculation for one leak at the aortic aspect and a denuded ventricular aspect endothelium for \( L_s = 107 \) \( \mu m \). To model endothelial denudation we set the endothelial permeability \( L_{e,v}^* \) and the ventricular subendothelial intima’s \( K_{p,v}^* \) to a large number (here, 10,000) times their intact endothelial value in Table 1 and require \( C/R_{z} \gamma_z = 1 \) at \( z = 1 \) instead of Eqs. 22 and 23 as the boundary condition at \( z = 1 \), corresponding to direct contact of the middle layer with plasma fluid. As before, there are no free parameters. Curve 1 fits all but points 7–9 (counting from \( z = 0 \)) well. This theoretical curve, as well as all others that we have produced with this model, even with different parameters, is always convex, unlike data points 7–9 that seem to exhibit an inflection point.
APPENDIX: SOLUTION METHODS

The governing equation for the filtration model, Eq. 6, is a 2D Laplace equation that has a straightforward analytical solution in terms of Bessel functions. However, the matching conditions at the two interfaces make this analytic solution cumbersome. More importantly, because the leaky junction’s width ∆R = 35 nm (43), 10⁻⁴ of the model’s radial dimension ξ, it would be necessary to retain an impractically large number of terms in the Bessel function expansion of the solution to resolve variation over the relevant length scale of the junction width to obtain a numerically accurate result. (Proposed techniques to overcome this problem described in Ref. 41 are in error because the junctional basis functions proposed there do not reliably form a complete orthogonal set; in practice, their use apparently always retains the zeroth function, the constant.) Finally, the resulting complex velocity profile, in any case, requires a direct form a complete orthogonal set; in practice, their use apparently always retains the zeroth function, the constant.) Finally, the resulting complex velocity profile, in any case, requires a direct numerical solution of the 2D convection-diffusion equation, Eq. 14, for the mass transport problem, and so a discretization is necessary for that part of the problem. For all of the above reasons, we use a direct discretization approach, finite differences, for the filtration part of the problem as well.

To integrate the filtration and the convection-diffusion problems and to simplify the calculations, one uses a single set of grid points for both. Because of the likely very sharp pressure and concentration gradients in a small region near the leaky junction, a region comparable to the width (∼30 nm) of the junction itself, and due to the much smaller thickness of the valvular intima, ∼200 nm for rat and rabbit (46), than that of the valvular leaflet, it is clearly advantageous to use variable grid sizes. The grid size is smallest, 30 nm, near the leaky junction and gradually increases in the r direction to a maximum of 8 µm far from the leak, since the pressure and concentration curves are extremely steep near the leaky junction and then become much flatter far away from it. In the z-direction, the grid size in two layers of valvular intimae is much smaller, 20 nm, than that in the middle layer, 4 µm. The central difference formulae for the first- and second-order derivatives for variable grid sizes are different from those under the classic uniform grid sizes and are defined as

\[ f'(u) = \frac{f(u + h_2) - f(u - h_1)}{h_1 + h_2} \]  
\[ f''(u) = \frac{h_1 f(u + h_2) + h_2 f(u - h_1) - (h_1 + h_2) f(u)}{\frac{1}{2} h_1 h_2 (h_1 + h_2)} \]

\[ \frac{\partial P_i}{\partial r} \]  
which takes indeterminate form

\[ \frac{\partial P_i}{\partial r} \]  
there. By Maclaurin’s expansion,

\[ \frac{\partial P_i}{\partial r} \bigg|_{r=0} + r \frac{\partial^2 P_i}{\partial r^2} \bigg|_{r=0} + \frac{r^2}{2} \frac{\partial^3 P_i}{\partial r^3} \bigg|_{r=0} + \ldots, \quad i = 1, 2, 3 \]

but according to Eq. 7,

\[ \frac{\partial P_i}{\partial r} \bigg|_{r=0} = 0 \]

So

\[ \lim_{r \to 0} \frac{\partial P_i}{\partial r} = \frac{\partial^2 P_i}{\partial r^2}, \quad i = 1, 2, 3 \]

Thus Eq. 6 at \( r = 0 \) can be replaced by

\[ 2 \frac{\partial^2 P_i}{\partial r^2} + \frac{\partial^3 P_i}{\partial r^3} = 0, \quad i = 1, 2, 3 \]

After solving the filtration model, one substitutes the velocity fields’ solutions into the macromolecular transport equation (Eq. 14). Just as Eq. 6, after inserting Eqs. 4 and 16b. Eq. 14 contains two terms,

\[ \frac{1}{r} \frac{\partial C_i}{\partial r} \]

and

\[ \frac{U_i}{r} \]

that are singular at \( r = 0 \), and which resolve to

\[ \lim_{r \to 0} \frac{1}{r} \frac{\partial C_i}{\partial r} = \frac{\partial^2 C_i}{\partial r^2}, \quad i = 1, 2, 3 \]

\[ \lim_{r \to 0} \frac{U_i}{r} = -K_{ci} \lim_{r \to 0} \frac{\partial P_i}{\partial r} = -K_{ci} \frac{\partial^2 P_i}{\partial r^2}, \quad i = 1, 2, 3 \]

where the solution to the filtration model has determined

\[ \frac{\partial^2 P_i}{\partial r^2} \]

We use the Hopscotch method, a fast second-order partial differential equation solver, to solve the boundary value problem for the solute transport. This method, a type of finite difference method described in Ref. 13, has been shown to be fast, efficient, and unconditionally stable.

Since the exact analytic or an a priori accurate solution to the two-dimensional problem is unknown, one adopts different refinements/sets of mesh points with decreasing grid sizes to test the accuracy until the differences between two sequential computations are small enough. As we have shown, the 2D model for the case of no leaks in either endothelial aspect reduces to a 1D model whose mass transport solution more easily lends itself to the standard Crank-Nicolson implicit finite difference method (30). We further verify the accuracy of the 2D solution by comparing, in this limiting case, with the 1D Crank-Nicolson solution and find excellent agreement.

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