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Modeling of Aquaporin1-Mediated Transmural Water Transport and the Resulting Oncotic Paradox

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Abstract—**The earliest observable prelesion event in atherosclerosis, macromolecular transport across the vessel wall, occurs via advection by transmural pressure-driven water transport, characterized by the hydraulic conductivity (Lp), defined as the ratio of water flux to the transmural pressure difference. The discovery of the presence of aquaporin-1 (AQP) in aortic endothelial cells suggests a new possibility of water transport across the endothelial cell (EC), alongside the generally accepted paracellular route. In this study, we propose a new filtration theory to explain the experimentally observed pressure-dependent effect of AQP-blocking on the Lp of rat aorta. However, given the isotonic lumen, this AQP-mediated pure water inflow into the arterial subendothelial intima (SI) should set up an oncotic pressure gradient that opposes the ΔP-driven flow through the cell. How then could trans-AQP flow persist for many hours, as indicated by chemical blocking of AQP experiments? To resolve this paradox, we have extended our filtration theory to also include the mass transfer of oncatically active small solutes like albumin. This addition non-linearly couples the mass transfer, the fluid flow and the wall mechanics. We employ finite difference methods to simultaneously solve the filtration and mass-transfer problem as a long-time solution of an unsteady problem. Our results agree well with the experimental data and suggest that AQPs contribute about 30% to the phenomenological endothelial Lp. We have also found that, due to media filtration, at steady state, the albumin concentration in the SI is in fact higher than in the glycocalyx. This results in higher osmotic pressure in the SI, which drives the fluid flow into the SI from the luminal side of the EC and not the other way around. Controlling endothelial Lp, via AQP expression, might serve as a future therapeutic target to inhibit pre-atherosclerotic events.**

I. Introduction

THE transport of macromolecules like low-density lipoprotein (LDL) cholesterol across the blood vessel wall, eventually leading to atherosclerotic lesions, has been the focus of intense theoretical and experimental studies [1]. This macromolecular transport process is known to occur due to advection by transmural pressure (the pressure difference between inside and outside the vessel) driven water transport, characterized by the hydraulic conductivity Lp, the ratio of the transmural water flux to the driving pressure difference [2,3]. Water, being very small, can easily cross the endothelium via inter-endothelial cell (EC) junctions and enter the vessel wall (paracellular route), while macromolecules can only enter through rare, transient 'leaky' junctions, associated with ECs that are either dying or dividing. It is crucial to understand the details of transmural water transport because, on one hand, it advects LDL into the arterial subendothelial intima (SI) and spreads it there, thereby giving it the chance to bind to extra cellular matrix (ECM) and possibly trigger the start of lesion formation. On the other hand, it dilutes LDL's local concentration, thereby likely slowing its ECM-binding kinetics, and washes not-yet-bound lipid further into the wall. Besides the paracellular route, the presence of highly specific water channel proteins like aquaporin-1 (AQP) in a variety of epithelial and endothelial cells, suggests a new possibility of water transport via AQPs (transcellular route).

Preliminary data from our group show that bovine aortic ECs express AQPs in cultured monolayers and that rat aortic ECs express AQPs in excised whole vessels. Chemically blocking AQPs using $HgCl_2$ or knocking down AQP expression showed a significant reduction in monolayer Lp [4]. Moreover, blocking EC AQPs reduced the Lp of excised vessel by ~32%, 11% and 5% at 60, 100 and 140 mmHg, respectively, suggesting significant AQP-mediated transcellular flow [4]. One mystery that our theory addresses is why this drop is pressure dependent. Although the percentage drop does not depend on pressure, Lp itself was found to be pressure-independent starting at as low as 60 mmHg upon AQP blocking. We have developed a model for filtration through fenestral pores that, for the first time, considers the role played by AQPs in modulating the total hydraulic conductivity of an intact arterial wall with changes in transmural pressure. Our hypothesis suggests that blocking the AQPs will decrease the number of available pathways for water transport, thereby decreasing the intimal pressure $\langle P_i \rangle$ at fixed ΔP. Thus, there is larger force per unit area [P_L (lumen pressure) – $\langle P_i \rangle$] acting on the endothelium that can compress the intima and lead to fenestral blockage at lower overall ΔP.

Even though this filtration theory explains our Lp observations extremely well, the transmural pressure-driven trans-AQP mediated water flow presents an oncotic paradox. The idea is that normal EC junctions transport isotonic fluid, i.e., water and small solutes (e.g. albumin) responsible for oncotic pressure. In contrast, AQPs specifically transport

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pure (hypotonic) water, excluding nearly everything else. This pure water inflow should both raise the albumin concentration on the lumen side and lower it on the SI side of the EC, thereby setting up an osmotic pressure gradient that acts against the ΔP-driven flow through the cell and prevents the SI from getting more hypotonic. This begs the question, how can the vessel maintain a steady trans-AQP water flow given its effect on intima tonicity? To understand the effect of such oncotic gradients on the overall transport and to resolve this paradox, we develop a model for water and small solute transport across the glycocalyx layer, across endothelial interface and through the entire wall. Here, we combine our filtration theory with the mass transfer of albumin and solve the two problems simultaneously using finite difference methods. This problem is nonlinear – since flow couples to mass transfer, which, in turn affects flow - and thus presents significant calculational challenges.

II. Mathematical Model

Figure 1 shows a representative local periodic wall unit of a circular cylinder with an EC of radius ξ^*. An internal elastic lamina (IEL) fenestral pore of radius r_f^* is at the unit's center. Fluid enters the SI from the lumen along the wall unit's perimeter, which represents the normal EC junction, and through the EC via AQPs. We only consider the water flow entering the SI through normal clefts (of width ΔR^*) because they vastly outnumber the leaky clefts by a factor of 2000-6000 and account for the overwhelming majority of the water flow across the endothelium. Figure 1 greatly exaggerates the vertical SI scale, which is of the order of 0.2–0.5 μm in healthy rat aorta, and the placement of a fenestra beneath the center of the cell is also an obvious idealization. The IEL is treated as an impenetrable barrier of zero thickness except for its fenestral opening. A glycocalyx layer of thickness L_g^* sits on top of the EC. It plays a key role in the transport of small solutes through the vessel wall. The SI, under a non-deformable endothelium, can be compressed by pressure loading from an SI thickness of L_{i0}^* to L_i^*. Water entering the SI through intercellular junctions flows laterally (i.e., parallel to the endothelium) in the SI, joined by water entering through the EC AQPs, and enters the media through the IEL fenestra. U^* and W^* are water velocities in the radial and vertical directions respectively. The liquid is incompressible. Since the tissue is a porous medium, flow through it satisfies Darcy's law. Combining these two facts, we get a set of coupled Laplace's equations $\nabla^2 P = 0$ in cylindrical co-ordinates for each domain: namely, glycocalyx, SI and media. We invoke axisymmetry at the center and periodicity at the edge of periodic wall unit. The lumen and adventitia are assumed at fixed constant pressures of (pressure units chosen to give) 1 and 0, respectively. Pressure and velocity are continuous in the fenestral hole and there is zero fluid flux (due to impermeability) through the IEL. This defines a mixed-boundary value problem. The water flux $W^* = L_{P(EC)} \cdot (\Delta P - \sigma_{EC} \Delta\pi)$ across the EC and the normal junction $W^* = L_{P(nj)} \cdot (\Delta P - \sigma_{nj} \Delta\pi)$, along with the velocity continuity in the junction, add one more mixed-boundary condition to the problem, where σ_{EC} and σ_{nj} are osmotic reflection coefficients of albumin through AQPs and normal junction respectively.

The osmotic component $(\Delta\pi)$ that affects the water flux entering the SI is governed by the concentration of oncotically active albumin [5]. We calculate the albumin distribution by solving a convection-diffusion equation $(f/\gamma) V \cdot \nabla C = D \nabla^2 C$ in each domain (f is the retardation coefficient, γ the volume fraction available for albumin and D the diffusivity of albumin). The concentration of albumin is assumed to be same at the lumen and adventitia. We use concentration and solute flux continuity in the fenestra and a zero flux condition over the IEL. The solute flux across the EC and normal junction is governed by the permeabilities of

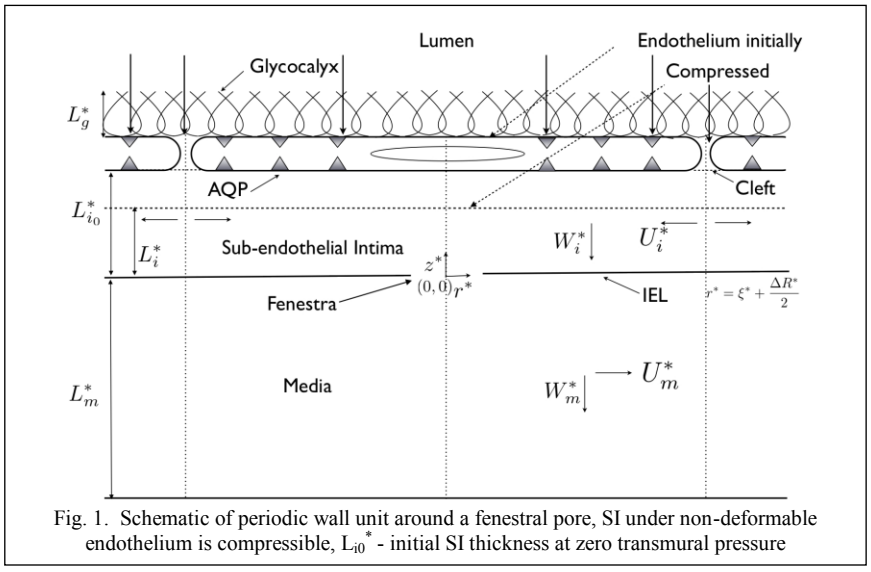

Fig. 1. Schematic of periodic wall unit around a fenestral pore, SI under non-deformable endothelium is compressible, L_{i0}^* - initial SI thickness at zero transmural pressure

the EC and junction, respectively. The required velocities in these equations are obtained from the filtration problem.

Since the mass-transfer and filtration problems are coupled, we simultaneously solve the combined steady state problem as the long-time solution of the unsteady problem using a finite difference method. We use parameters from the literature, mostly from experiments. Due to significant mismatches in the problem's length scales, we use a highly non-uniform mesh, especially near the fenestra and the junction. The governing equations and boundary conditions are discretized using second order difference formulae. This discretization procedure leads to a linear system of algebraic equations whose number equals the total number of mesh points. We solve the set of equations representing the three domains simultaneously using Matlab (Mathworks Inc). Numerical accuracy is achieved using successive mesh refinement. Once the pressure distribution inside the wall is known, the overall fluid flux gives the hydraulic conductivities at different transmural pressures.

III. Results and Discussion

A. Hydraulic conductivity:

Figure 2 shows the hydraulic conductivities of an aortic wall for functioning (or open) and blocked AQPs at different transmural pressures, with various assumed fractions of AQP contributions, and compares these predictions with Nguyen's experimental data [4]. In the case of open AQPs, the $L_P(\Delta P)$ curve becomes flatter and shifts to right with increasing AQP fraction (results not shown). The higher intimal pressures resulting from increased number of AQPs result in less force acting on the endothelium. Hence, with more AQPs, one needs higher overall tramsmural pressures before the force acting on the endothelium reaches the critical pressure

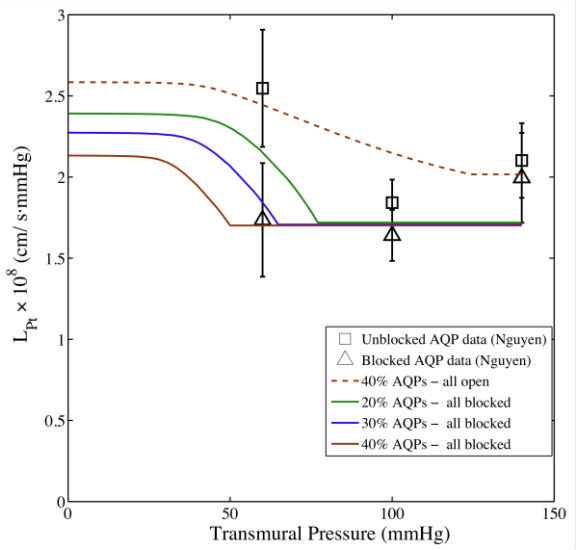

Fig. 2. Effect of AQP blocking on the hydraulic conductivity of rat aorta. Lines – model predictions, symbols – experimental data [4], squares – functioning AQPs, triangles – blocked AQPs (using $HgCl_2$ as a chemical blocker).

needed to fully compress the SI, after which stiffer collagen fibers do not allow any further compression of the SI, and the hydraulic conductivity remain almost constant. However, when AQPs are blocked, Lp decreases and the $L_P(\Delta P)$ curve shifts to left, meaning that the SI achieves its maximum compression limit at much lower transmural pressures. Upon AQP blocking, the average pressure in the SI is reduced, thereby increasing the force per unit area acting on the endothelium and compressing the SI at much lower overall transmural pressure. Our model also predicts that the force acting on the endothelium at 60 mmHg with functioning AQPs is same as that at 44 mmHg when the AQPs are blocked. In other words, in the case of blocked AQPs, the force acting on the ECs is much higher than the force on ECs with their AQPs intact. This suggests that AQP up-regulation might lead to higher EC Lps, which might keep the SI from compressing at physiological pressures. This might result in increased dilution of lipid that has entered the SI through the leaky junctions, which could slow the kinetics of its binding to SI extracellular matrix, the process believed to trigger lesion formation.

B. Concentration of albumin & osmotic pressure:

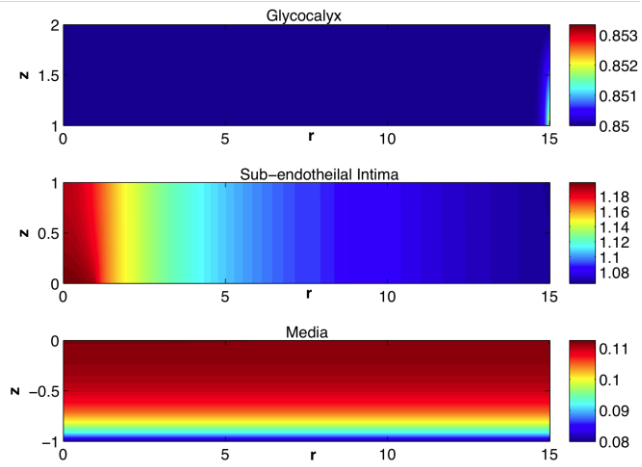

Fig. 3. Non-dimensionalized albumin concentration in glycocalyx, SI and media. Note that colorbar is different for each figure.

Figure 3 shows the absolute concentration of albumin (based on the available volume fraction) in each of the three regions. Our results show that, since the media retardation coefficient ~ 0.3 (derived from ref. 3), the media acts to strongly filter the transport of the solute albumin relative to the water solvent, causing a build-up of albumin in the media that backs up into the SI by the time steady state is achieved. In contrast to our naïve suggestion above, this increased albumin concentration results in *higher* osmotic pressures in the SI than in the glycocalyx (see fig. 4), which drives the water flow into the SI from the luminal side of the EC, and not the other way around. Thus our theory predicts that the albumin concentration gradients across the EC rather favors the transmural pressure driven flow, consistent with

experimentally observed steady transmural flows, and thereby resolves an interesting oncotic paradox. In addition, the Lp's predicted without considering the osmotic effects are found to be at least 8-10% higher in the dynamic range of 60-100 mmHg. Thus osmotic gradient mask the true wall hydraulic conductivity to this extent.

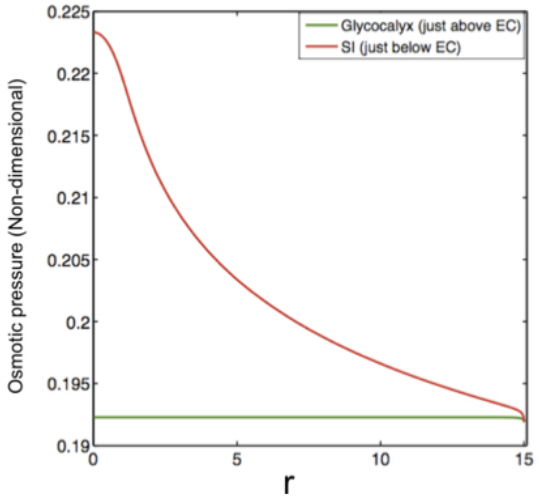

Fig. 4. Non-dimensionalized osmotic pressure above and below the EC.

IV. Conclusion

We have presented a model for the filtration through fenestral pores and considered, for the first time, the role played by AQPs in modulating the total hydraulic conductivity of an intact arterial wall. Our model predicts large drops in Lp upon AQP blocking that agree well with Nguyen's [4] experimental observations of the effect of AQP blocking on Lp at different transmural pressures, supporting the hypothesis that AQPs indeed play a significant role in overall transport across the arterial wall. We have also found that AQP blocking, when done at pressures where the SI would otherwise be uncompressed, can cause the SI compression and initiate fenestral blocking. This suggests that AQP up-regulation, and the resulting reduced force, might keep the SI uncompressed and increase the vessel wall's hydraulic conductivity. Thus, increased Lps may be able to dilute LDL's local concentration and this might suggest an avenue for slowing down the early progress of atherosclerosis. Our mass-transfer theory, non-linearly coupled to the filtration problem, resolves an interesting oncotic paradox. Even though oncotic gradients are normally assumed negligible across the walls of large arteries, we find that they seem to indeed play a role *within* those vessel walls. Given AQP's presence in the high-pressure cardiac endocardium, heart, lung and renal epithelia, resolving such flow paradoxes might have important implications.

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