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Computer Simulation and Experimental Analysis of LDL transport in the Arteries

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Abstract—**Atherosclerosis develops from oxidized low-density lipoprotein molecules (LDL). When oxidized LDL evolves in plaque formations within an artery wall, a series of reactions occur to repair the damage to the artery wall caused by oxidized LDL. Macrophages accumulate inside arterial intima, they started to collect oxidized LDL and form foam cells. Smooth muscle cells accumulate in the atherosclerotic arterial intima, where they proliferate and secrete extracellular matrix to form a fibrous cap.**

In this study, experimental model of LDL transport on the isolated blood vessel from rabbit on high fat diet after 8 weeks is simulated numerically by using a specific model and histological data. The 3D blood flow is governed by the Navier-Stokes equations, together with the continuity equation. Mass transfer within the blood lumen and through the arterial wall is coupled with the blood flow by the convection-diffusion equation. LDL transport in lumen of the vessel is described by Kedem-Katchalsky equations. The inflammatory process is solved using three additional reaction-diffusion partial differential equations.

Matching of histological rabbit data is performed using 3D histological image reconstruction and 3D deformation of elastic body. Computed concentrations of labeled LDL of 5.2 % and macrophages distribution of 4.2% inside the media are found to be in good agreement with experimental results. This simulation study provides a useful tool for understanding and prediction of LDL transport through the arterial wall and evolution of atherosclerotic plaques.

I. Introduction

It is well known the position of the endothelium at the interface between blood and vessel wall with main role as a barrier to the transvascular convection and diffusion of blood-borne macromolecules. The endothelial cells lining the blood vessels are flattened and elongated with nuclei that protrude into the lumen. They form a layer that prevents blood cell interaction with the vessel wall with a critical role in mechanics of blood flow, regulation of coagulation, leukocyte adhesion, and vascular smooth muscle cell growth. Damaged endothelium induces physiological and pathological changes [1], [2] such that decreased integrity of the endothelial barrier permits easier macromolecular transport into the intima [3].

Inflammatory process starts with penetration of low density lipoproteins (LDL) in the intima. This penetration, if too high, is followed by leucocyte recruitment in the intima. This process may participate in formation of the fatty streak, the initial lesion of atherosclerosis and then in formation of a plaque [4].

There are three major categories of LDL transport models. The simplest models are wall-free models, in which the arterial wall is substituted by a simplified boundary condition. Rappitsch and Perktold [5] and Wada and Karino [6] applied these models for the analysis of the macromolecular transport in the arterial wall. A more realistic approach is lumen-wall models, where there is coupling of the transport within the lumen and the wall, Moore and Ethier [7], Stangeby and Ethier [8]. Also there are multilayer models, which break the arterial wall down into several layers and model the transport within the wall, either at the microscopic [8]–[11] or macroscopic [12]–[16] levels. There are no so many numerical studies which rely on real experimental data for LDL transport.

In this paper we firstly described experimental setup for the LDL transport into the blood vessel wall in the isolated rabbit carotid artery under physiologically relevant constant pressure and perfusion flow on rabbit with 6 weeks high fat diet. Then we have shown computational procedure for mass transport of LDL through the wall and the simplified inflammatory process which is coupled with the linearized Navier-Stokes equations, the Darcy equation for model blood filtration and Kedem-Katchalsky equations [17],[18] for the solute and flux exchanges between the lumen and the intima. The following is system of three additional reaction-diffusion equations for the inflammatory process and lesion growth model in the intima. The next section presented numerical simulation and comparison with some initial experimental animal results of LDL transport and histological analysis.

II. Experimental Setup

A. Blood vessel preparation

Ex vivo blood vessels experiments of LDL transport were performed on the isolated rabbit a. carotid comm. All experiments were performed according to the Animals Scientific procedures Act 1986 (UK) and local ethical guidelines. New Zealand White rabbits of both sex weighing 3.5-4 kg were anesthetised using Ketamine (Laboratorio

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Sanderson, Santiago, Chile), 4-6 mg per kg of body weight. Blood vessel was excised and placed in the water bath. Cannulas with equally matched tip diameters (2mm) were mounted at proximal (cardial) and distal (cranial) ends of the blood vessel. The lumen was perfused with Krebs-Ringer physiological solution (KRS), using the peristaltic pump at 1 ml/min. The perfusate was continuously bubbled with a 95% O_2, and 5% CO_2 with the pH adjusted to 7,4 at 37 C.

The distal cannula was connected to the resistance changing device. Perfusion pressure was measured with perfusion transducer.

The blood vessel was stretched to its approximate in vivo length. The outer diameter of the blood vessel was measured using digital camera and originally developed software. The blood vessel wall thickness was measured at the end of each experiment, using light microscope and microscopically graduated plate. The blood vessel was considered to be viable if it contracted when 25 mMKCl was added to the bath, as well as if the presence of functional endothelium was verified by dilation with Ach (1μM) at the end of experiment.

Fig. 1.Setting for *ex vivo* blood vessels experiments: 1. Pressure and temperature A/D converter, 2. Peristaltic pump, 3. Heater thermostat, 4. Rapid infusion pump (RIP), 5.Automatic sampler, 6.Resistance changing device (RCD), 7. Control unit for RIP, 8. Control unit for RCD, 9. Syringe infusion pump, 10. Water bath, 11.Heating stabiliser, 12.PC, 13.Digital camera.

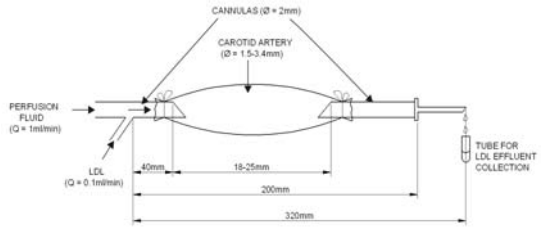

Fig 2. Schematic presentation of the isolated blood vessel segment in the water bath

B. Rapid dual –isotope dilution method

This method measures the relative extraction (uptake) of the ^{125}I-LDL (test molecule) in relation to ^{99m}Tc-Nanocis (as an extracellular referent tracer) as its transit through the blood vessel [19]-[21].

C. ^{125}I-LDL (test tracer) and ^{99m}Tc-Nanocis (referent tracer) dilution profiles in the isolated blood vessels

^{125}I-Low density lipoprotein was prepared to obtain ^{125}I-LDL solution containing 50μl commercially obtained ^{125}I-LDL (Biomedical Technologies Inc) and 950μl physiological buffer. Then, ^{125}I-LDL solution was used to prepare ^{125}I-LDL standard solution which contained 50μl ^{125}I-LDL solution and 300 μl physiological buffers. From ^{125}I-LDL standard solution, three aliquots (100 μl) were prepared and their activity was measured using gamma counter (Wallac Wizard 1400). Mean value of the aliquots radioactivity was 373916.1±1777.02 cpm (mean ± SE). *Reagents*: ^{125}I-Low density lipoprotein (Biomedical Technologies Inc, USA, Catalog no. BT-903); specific activity: 0.102μCi/ml; quantity: 525 μCi/2ml.

The ^{125}I-LDL uptake is derived from the difference between the ^{99m}Tc-Nanocis value and that of ^{125}I-LDL recovery in each sample

III. Computational Procedure

We firstly described mass transfer problem for LDL transport through the wall and then a continuum based approach for plaque formation and development in three-dimension is described. The governing equations and numerical procedures are given. The blood flow in lumen domain is simulated by the three-dimensional Navier-Stokes equations, together with the continuity equation

$$-\mu\nabla^2 u_l + \rho(u_l\cdot\nabla)u_l + \nabla p_l = 0 \quad (1)$$

$$\nabla u_l = 0 \quad (2)$$

where $\boldsymbol{u_l}$ is blood velocity in the lumen, p_l is the pressure, μ is the dynamic viscosity of the blood, and ρ is the density of the blood [22].

Mass transfer in the blood lumen is coupled with the blood flow and modeled by the convection-diffusion equation as follows

$$\nabla\cdot(-D_l\nabla c_l + c_l u_l) = 0 \quad (3)$$

in the fluid domain, where c_l is the solute concentration in the blood lumen, and D_l is the solute diffusivity in the lumen.

Mass transfer in the arterial wall is coupled with the transmural flow and modeled by the convection-diffusion-reaction equation as follows

$$\nabla\cdot(-D_w\nabla c_w + kc_w u_w) = r_w c_w \quad (4)$$

in the wall domain, where $c_w$is the solute concentration in the arterial wall, D_w is the solute diffusivity in the arterial wall, K is the solute lag coefficient, and r_w is the consumption rate constant.

LDL transport in lumen of the vessel is coupled with Kedem-Katchalsky equations:

$$J_v = L_p(\Delta p - \sigma_d\Delta\pi) \quad (5)$$

$$J_s = P\Delta c + (1-\sigma_f)J_v\bar{c} \quad (6)$$

where L_p is the hydraulic conductivity of the endothelium, Δc is the solute concentration difference across the endothelium, Δp is the pressure drop across the endothelium, $\Delta\pi$ is the oncotic pressure difference across the endothelium, σ_d is the osmotic reflection coefficient, σ_f is the solvent reflection coefficient, P is the solute endothelial permeability, and $\bar{c}$ is the mean endothelial concentration.

The first term in Kedem-Katchalsky equations $P\Delta c$ of the right hand side in (6) defines the diffusive flux across the endothelium, while the second term $(1-\sigma_f)J_v\bar{c}$ defines the convective flux. Here we do not neglect the convective term. Only the oncotic pressure difference $\Delta\pi$ is neglected because

of decoupling the fluid dynamics from solute dynamics. We are using the incremental-iterative procedure to treat the convective diffusion terms for LDL transport.

The atherosclerotic process starts with the accumulation of LDL in the intima, where part of them are oxidized and become pathological. In order to remove the oxidized particles, circulating immune cells (*e.g.* monocytes) are recruited. Once in the intima, the monocytes differentiate and become macrophages that phagocyte the oxidized LDL. Fatty macrophages then transform into foam cells. Foam cells are responsible for the growth of a subendothelial plaque which eventually emerges in the artery lumen.

The inflammatory process was solved using three additional reaction-diffusion partial differential equations [23]:

$$\begin{aligned} \partial_t Ox &= d_1 \Delta Ox - k_1 Ox \cdot M \\ \partial_t M + div(v_w M) &= d_2 \Delta M - k_1 Ox \cdot M + S/(1-S) \\ \partial_t S &= d_3 \Delta S - \lambda S + k_1 Ox \cdot M + \gamma (Ox - Ox^{thr}) \end{aligned} \quad (7)$$

where Ox is the oxidized LDL in the wall, M and S are concentrations in the intima of macrophages and cytokines, respectively; d_1,d_2,d_3 are the corresponding diffusion coefficients; λ and γ are degradation and LDL oxidized detection coefficients; and v_w is the inflammatory velocity of plaque growth, which satisfies Darcy's law and continuity equation [24]:

$$v_w - \nabla \cdot (p_w) = 0 \quad (8)$$

$$\nabla v_w = 0 \quad (9)$$

in the wall domain. Here, p_w is the pressure in the arterial wall.

In order to make plaque formation and development algorithm and to connect blood flow simulation with bioprocess modelling the 3D mesh moving algorithm and ALE (Arbitrary Lagrangian-Eulerian) formulation for fluid dynamics is applied [25].

IV. Results

The aim of our experiment is to determine distribution of accumulated ^{125}I-LDL radioactivity in the different segments of the isolated blood vessel. Specific software for 3D reconstruction of lumen domain and carotid wall artery was developed. Computer model of the artery is considered as a simple straight tube. The diameter of artery was D=0.0029m, the mean velocity U_0=0.24m/s, dynamics viscosity μ=0.0035Pas, density ρ=1050 kg/m^3. The transmural pressure under normal physiological condition was taken as 70 mmHg.

Histological images are shown in Fig. 3. The labeled LDL is localized in the white zones inside media which is probably due to destroyed radioactive LDL of tissue. Polylines around media are segmentation lines produces by in-house image processing software. Matching of histological data and computational simulation is presented in Fig. 4.The process of matching histological images was done by 2D deformation of each histological cross-section in order to keep the internal lumen approximately cylindrical shape. The maximum LDL was found at distal part of the carotid artery segment at 3.5 mm from entry segment. Afull three-dimensional finite element analysis was performed using our in-house finite element code in order wall shear stress and function of permeability for the wall. Oxidized LDL, macrophages and cytokines distribution is presented in Fig. 5. Diagrams of wall LDL, oxidized LDL, macrophages and cytokines inside wall are shown in Fig. 6. Experimental LDL transport of 5.2% was fitted with specific nonlinear least square analysis [26] in order to get numerical parameters. The fitted numerical parameters are given in Table 1.

Fig. 3. Histological data (numbers on photos indicate distances from entry carotid artery in millimeters). White zones inside media denote labeled LDL localization. Polylines around media are segmentation lines produces by image processing software.

TABLE I
VALUES FOR CAROTID ARTERY EXPERIMENT

Symbol	Description	Values
ρ	Blood density, lumen	1000kg/m^3
μ	Blood dynamic viscosity	0.035 [*Pa s*]
D_l	Diffusivity, lumen	1.0e10^{-12} m^2/s
Dw	Diffusivity, wall	3.0e^{-12} m^2/s
r_w	Consumption rate, wall	-2.6x10^{-4}
d_1	Diffusivity for oxLDL, wall	10^{-7} m^2/s
d_2	Diffusivity for Macrophages, wall	10^{-7} m^2/s
d_3	Diffusivity for Cytokines, wall	10^{-7} m^2/s
k_1	Flux coefficient	1.9e^{-4} m^3/kg s
λ	Degradation cytokines coefficient	25 s^{-1}
γ	Detection oxLDL coefficient	1s^{-1}

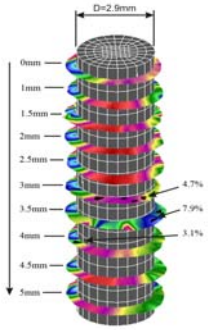

Fig. 4. Labeled LDL located in histology cross-section on each 0.5 mm for straight segment. Histology segments were obtained as deformable elastic rings opened from the current squeezed position to circle original tube. Black holes in these cross-sections show location of the labeled LDL. Percentages show labeled LDL area inside media and intima wall thickness.

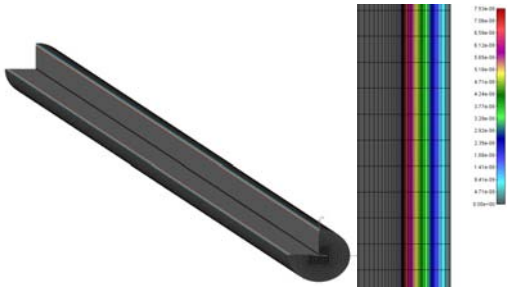

Fig. 5. Three-dimensional representation of the model (left panel).Macrophages distribution 4.2% from media (right panel).

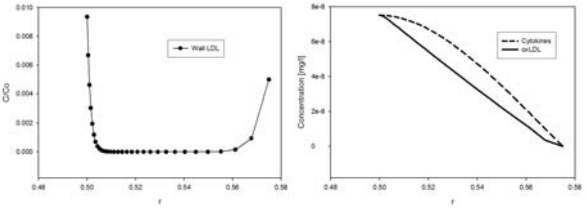

Fig. 6. Dimensionless wall LDL concentration profile in the media (upper); Oxidized LDL and Cytokines profiles in the media (bottom)

V. Discussion

In this study we compare our experimental results of LDL transport through the rabbit carotid artery together with histological analysis and computer model. A full three-dimensional model of LDL transport as well as plaque initiation is coupled with the Navier-Stokes equations and continuity equation. We used Darcy law for model blood filtration and Kedem-Katchalsky equations for the solute and flux transfer. The arterial wall permeability was fitted with our experimental results of LDL transport. All parameters for computer model were fitted with nonlinear least square procedure. Matching of labeled LDL location between experimental and computer model shows a potential benefit for future prediction of this complex process using computer modeling.

VI. Conclusions

The transport of LDL in the arterial wall, coupled with macrophages and oxidized LDL distribution has been investigated experimentally and using computer simulation. The material property of the arterial wall are treated with fitting procedure in order to match getting experimental results with histological data and isotope dilution method. The numerical results for LDL transport of 5.2% and macrophages distribution of 4.2% inside the media are found to be in good agreement with those from experiment.

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