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Modelling LDL accumulation in the case of endothelial dysfunction

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Abstract

The endothelium is responsible to keep the normal homeostasis of the vessels by regulating several biological and chemical mechanisms and responses. Nowadays it is evident that endothelial dysfunction is associated with an increased risk for plaque evolution. This work focuses on the modeling of LDL transport and accumulation in realistic geometries of coronary arteries in case of endothelial dysfunction. The Navier-Stokes equations and the convection-diffusion equations are utilized to simulate blood flow and LDL transport, respectively. Our model assumes shear stress dependent hydraulic conductivity, but also increased endothelial permeability in the case of endothelial dysfunction. For this purpose, the production of endothelial nitric oxide synthase (eNOS) is used for the calculation of nitric oxide (NO) synthesis. More specifically, the chemical reaction of NO production is modelled and the calculated concentration is taken into account to affect endothelial permeability. The obtained results demonstrate that endothelial dysfunction and NO concentration have an additional impact on LDL accumulation. Low concentration of NO, increases endothelial permeability resulting in an increased buildup of LDL molecules into the arterial wall. It appears that the LDL accumulation raises by up to 46% in case of endothelial dysfunction results which highlight the role of endothelial dysfunction on plaque evolution.

Keywords: LDL, accumulation, modeling, endothelial dysfunction

1. Introduction

Atherosclerosis is the most common cause of death in western societies. It is related to the thickening of the arterial wall and the consequent blockage of the lumen. The atherosclerotic plaque consists of lipids, monocytes, foam cells and smooth muscle cells. Atherosclerotic plaque development initiates with the accumulation of lipids and especially of the (low density lipoprotein) LDL in the arterial wall. Then, LDL is oxidized and stimulates an inflammatory process recruiting macrophages at the lesion. Foam cells are generated from monocytes which had endocytted oxidized LDL. Finally, SMC start to proliferate as a response to the inflammation and to the expressed chemokines and cytocines. Thus, today it is apparent that endothelial dysfunction promotes atherosclerosis while a normal function seems to have athero-protective capabilities

In recent years, it has become apparent that endothelium is not just a passive barrier, where molecules, cells and fluid from blood lumen penetrate the arterial wall considering that endothelium is simply a semi-permeable membrane. It is assessed experimentally, that endothelium plays an active role to keep the homeostasis of vessels [Celermajer D., 1997]. More specifically, endothelium controls cell (platelet or leucocyte) adhesion, its permeability, the thrombolysis, the vaso-constriction and dilation as well as many other parameters [Celermajer D., 1997].

An abnormal endothelial function will result in an increased endothelial permeability which is due either to a direct injury of the endothelial cells [Goodman R.B. et al, 2003] or to alterations in the gene expression on the endothelium. It has been showed that several factors such as thrombin, histamine, platelet activator factor (PAF), and Vascular endothelial growth factor (VEGF), which are released during the inflammatory responses of the arterial endothelium, increase its permeability by increasing the concentration of calcium (Ca_2^+), reactive oxygen species (ROS), and/or nitric oxide (NO) levels [Tiruppathi C. et al, 2003] – [Kubes P., 1995].

Nitric oxide (NO) has been found to play a significant role to the regulation of normal endothelial function [Khazaei M. et al, 2008.]. NO is synthetized from the semi-essential amino acid l-arginine and O_2 using the catalyst nitric oxide synthase (NOS) [Alderton W.K., 2001]. The basal level of NO, generated by endothelial NOS (eNOS) seems to maintain endothelial integrity, while high levels of NO produced by inducible NOS (iNOS) during inflammation, results to endothelial injury. The enzyme eNOS is localized on the endothelial cells and it has been shown that its expression is regulated from several factors including chemical molecules and the wall shear stress (WSS) [Fleming I., 2010]. NO has several implications on arterial physiology, since some experiments show that *eNOS−/−* knock-out animals are hypertensive or have increased endothelial permeability [Kubes P., 1995], [Whittle B.J.R., 1997].

Although several experimental studies have investigated the effect of endothelial dysfunction on plaque development there are only few computational studies that attempted to simulate the mechanism by which endothelial dysfunction contributes to atherosclerosis. Buerk [Buerk D.G., 2009] studied the interaction of NO and O_2 in case of endothelial dysfunction using a mathematical model that was based on finite elements analysis. Chen and Popel [Chen K. and Popel A.S., 2006] also proposed a theoretical analysis of the chemical reactions that take place during NO production. They modeled a network of chemical reactions and then validated their findings *in vivo*. Finally, Kar [Kar S.and Kavdia M., 2011] presented a model to calculate the NO production triggered by the biopterin concentration.

In this work, we propose a new computational approach to study the role of endothelial dysfunction on atherosclerotic plaque development. This uses realistic geometries of coronary arterial segments and attempts to assess the LDL transport in case of endothelial dysfunction.

For this purpose we model blood flow using the Navier-Stokes equations and calculate the LDL concentration solving the diffusion-convection equation. We simulate the chemical reaction of NO production from l-arginine and O_2. The endothelial dysfunction is modelled assuming that the expression of the eNOS gene is WSS dependent. Thus, in this manner, the permeability of the arterial wall depends on two variables: the eNOS production and the WSS dependent hydraulic conductivity, which is taken into account into the Kedem-Katchalsky equations.

2. Materials and methods

2.1 Geometry reconstruction

Coronary reconstruction of a left anterior descending artery is performed using intravascular ultrasound (IVUS) and bi-plane angiography data [Bourantas C., 2005]. More specifically, the obtained IVUS sequence is processed using active contours in order to segment the luminal and media-adventitia borders while the bi-plane angiographic images are used to extract the IVUS catheter path. Then, the detected borders are positioned onto the path and afterwards their absolute orientation is estimated using a well validated methodology. The final outcome of this approach is two set of point clouds of which one corresponds to the lumen and the other to the outer vessel wall.

2.2 Blood flow modelling

Blood is assumed to be Newtonian, while the flow is considered as laminar and incompressible. The Navier-Stokes equations are utilized to calculate the blood flow velocity and pressure:

$$\rho\left(\frac{\partial v_x}{\partial t}+v_x\frac{\partial v_x}{\partial x}+v_y\frac{\partial v_x}{\partial y}+v_z\frac{\partial v_x}{\partial z}\right)=-\frac{\partial p}{\partial x}+\mu\left(\frac{\partial^2 v_x}{\partial x^2}+\frac{\partial^2 v_x}{\partial y^2}+\frac{\partial^2 v_x}{\partial z^2}\right), \tag{1a}$$

$$\rho\left(\frac{\partial v_y}{\partial t}+v_x\frac{\partial v_y}{\partial x}+v_y\frac{\partial v_y}{\partial y}+v_z\frac{\partial v_y}{\partial z}\right)=-\frac{\partial p}{\partial y}+\mu\left(\frac{\partial^2 v_y}{\partial x^2}+\frac{\partial^2 v_y}{\partial y^2}+\frac{\partial^2 v_y}{\partial z^2}\right), \tag{1b}$$

$$\rho\left(\frac{\partial v_z}{\partial t}+v_x\frac{\partial v_z}{\partial x}+v_y\frac{\partial v_z}{\partial y}+v_z\frac{\partial v_z}{\partial z}\right)=-\frac{\partial p}{\partial z}+\mu\left(\frac{\partial^2 v_z}{\partial x^2}+\frac{\partial^2 v_z}{\partial y^2}+\frac{\partial^2 v_z}{\partial z^2}\right), \tag{1c}$$

$$\frac{\partial \rho}{\partial t}+\frac{\partial(\rho v_x)}{\partial x}+\frac{\partial(\rho v_y)}{\partial y}+\frac{\partial(\rho v_z)}{\partial z}=0, \tag{2}$$

where v_i are the velocity Cartesian components, p is the pressure, ρ is the blood density and μ is the blood viscosity.

At the inlet of the arterial segment, a transient blood flow profile is defined as it is shown in Fig. 1. At the outlet a constant zero pressure is applied. At the wall boundary, the transmural velocity is applied, defined by the Kedem-Katchalsky equations [Kedem O. and Katchalsky A., 1958].

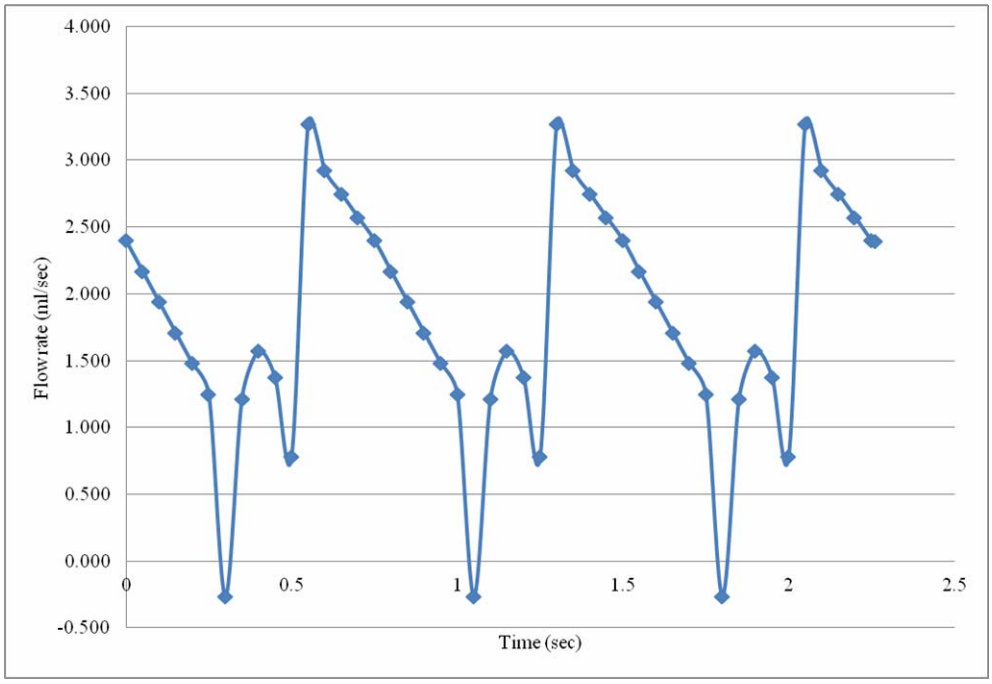

Fig. 1. Blood flow rate applied at the inlet of the arterial segment.

2.3. LDL transport

We assume that blood is a homogenous mixture with LDL, whose size does not affect the flow. LDL transport is modelled using the diffusion-convection equation:

$$\frac{\partial c}{\partial t}+v_x\frac{\partial c}{\partial x}+v_y\frac{\partial c}{\partial y}+v_z\frac{\partial c}{\partial z}=D\left(\frac{\partial^2 c}{\partial x^2}+\frac{\partial^2 c}{\partial y^2}+\frac{\partial^2 c}{\partial z^2}\right), \tag{3}$$

where c is the LDL concentration and D is the LDL diffusivity.

We assume constant LDL concentration equal to 4.1 mol/m^3, a value that corresponds to the LDL's upper normal limit. A convective flux is applied at the outlet of the arterial segment. At the endothelial boundary, solute flux from the lumen into the arterial wall is defined, expressed by the Kedem-Katchalsky equations.

2.4. Endothelial permeability

Two mechanisms for endothelial penetration are proposed. First, the Kedem-Katchalsky equations are used to model the endothelial permeability. Then we re-assess endothelial penetration assuming that its permeability depends also on the NO concentration. Both mechanisms are considered to be WSS dependent.

2.4.1 Kedem-Katchalsky equations

We assume that the endothelium is a semi-permeable biological membrane and we utilize the Kedem-Katchalsky equations [Kedem O. and Katchalsky A., 1958] to calculate the transmural velocity and solute flux across the endothelium:

$$Jv = Lp(\Delta p - \sigma_d \Delta \pi), \tag{4}$$

$$Js = P\Delta c + (1-\sigma_f) Jv\bar{c}, \tag{5}$$

where J_v is the transmural velocity and J_s is the solute flux through the endothelium, L_p is the hydraulic conductivity, Δp and $\Delta \pi$ are the pressure difference and the oncotic pressure difference across to endothelial membrane respectively, c is the solute concentration, and σ_d, σ_f are the reflection coefficients.

Furthermore, it is assumed that the hydraulic conductivity is WSS dependent. Based on the Sun's report [Sun N. et al, 2009], the relation of the hydraulic conductivity to the WSS is given by the following equation:

$$L_p(WSS) = 0.2077 \times 10^{-12} \ln(WSS + 0.015) + 3.1588 \times 10^{-12} \tag{6}$$

2.4.2 Endothelial dysfunction

To model the endothelial dysfunction, we model first the chemical reaction of the NO production from L-arginine and O_2 using the following formula:

L-arginine + $2O_2$ +1.5NADPH → *L-citrulline* + NO + 1.5$NADP^+$ +$2H_2O$.

This chemical reaction is catalyzed by eNOS and it can be transformed in the following mathematical equation using the Michaelis-Menten kinetics [Johnson K. and Goody R., 2011]:

$$NO = NO_{max} \text{ x } PO_2 / (PO_2 + K_m), \tag{7}$$

where NO_{max} is the maximum rate achieved by the system, at maximum substrate concentrations and the Michaelis constant K_m is the substrate concentration at which the reaction rate is half of NO_{max}. PO_2 is the partial pressure of oxygen, equal to 60 mmHg. In this simulation, NO_{max} is equal to 0.585 μmol/min/mg and K_m to 4.7 μM.

In the case of endothelial dysfunction we assume that the whole endothelium is dysfunctional and that the extent of the dysfunction is related to the local concentration of the produced NO which is a function of total eNOS concentration. Nevertheless, the expression of eNOS gene is found to by affected by the WSS [Wasserman S.M. et al, 2002], [Noris M. et al, 1992] and based on experimental data reported by Ishibazawa et al. [Ishibazawa A. et al, 2011] the relation between $|eNOS|$ concentration and WSS can be described using the following equation:

$$|eNOS| = 0.0033 \ln WSS + 0.0322. \tag{8}$$

It has been found that the inhibition of NO production causes an increase in the endothelial permeability. Roberts et al. [Roberts K.A. et al, 1997] demonstrated the NO concentration can affect endothelial permeability and LDL buildup. Warboys et al. [Warboys C. et al, 2010] attempted to quantify this association and found that LDL's permeability is two-fold increased after the inhibition of NO production. To our knowledge there is not enough quantitative data to create a realistic relation between WSS and LDL permeability and in this study we assumed that there is a linear inverse relation between endothelial permeability and NO concentration reduction.

3. Results

Figure 2(a) illustrates the WSS distribution at the arterial wall of the reconstructed coronary artery. The average WSS is 3.4 Pa. Panel (b) shows the areas with low WSS (0-2 Pa) which are about 6.2×10^{-6} m^2.

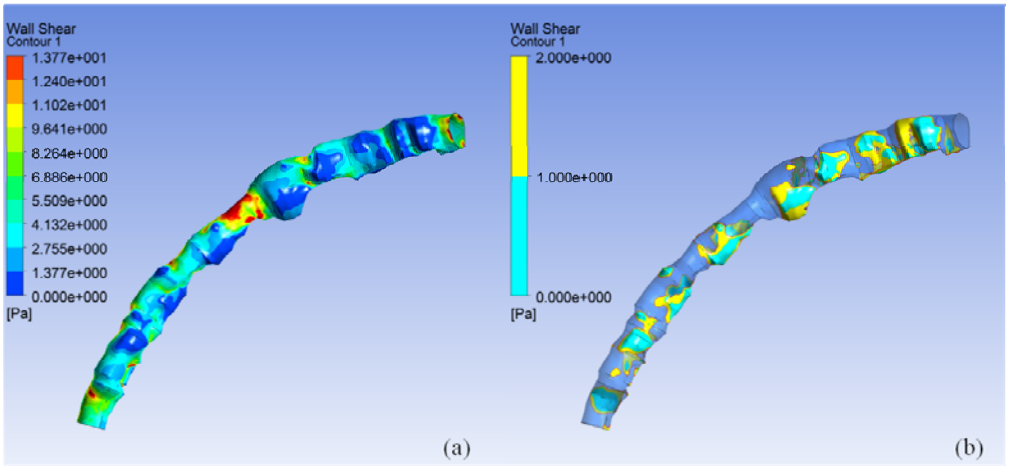

Fig. 2. (a) WSS at the coronary arterial segment, and (b) regions with low WSS depicted with a yellow and light-blue color (0-2 Pa).

Based on our assumption about the up-regulation of eNOS gene by WSS, we expect increased NO concentration at the regions with high WSS and low NO concentrations in regions with reduced WSS. This results in an inhomogeneous distribution of NO concentrations in the reconstructed vessel that is shown in Fig 3.

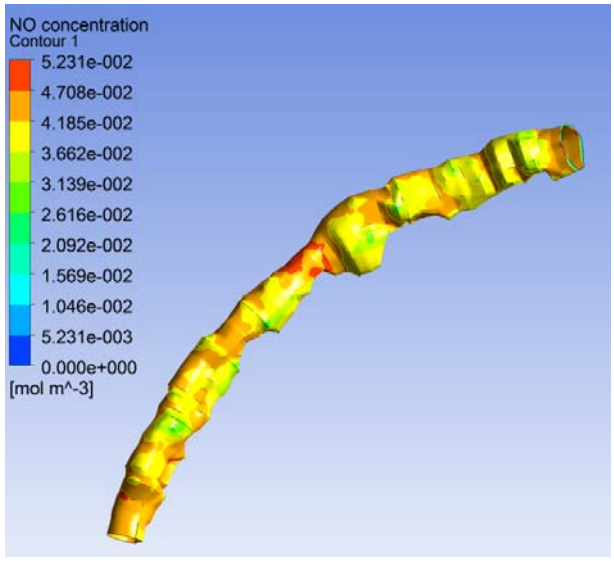

Fig. 3. NO concentration at the arterial segment. High concentration is found at high WSS regions.

Finally, Fig. 4 portrays in a color coded map the accumulation of the LDL in the arterial wall. As it would have been expected the LDL molecules are increased in regions with low WSS.

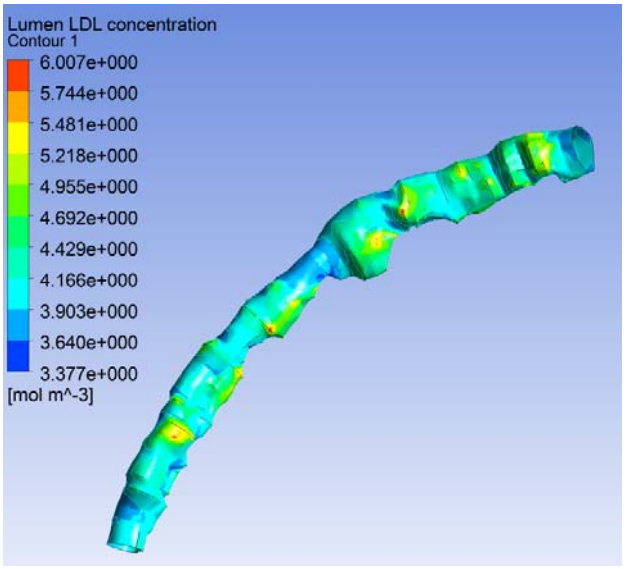

Fig. 4: Calculated LDL concentration on the arterial wall.

4. Discussion

We model LDL transport in the setting of endothelial dysfunction. In this study it was assumed that the LDL penetration of the endothelium depends not only on WSS related hydraulic conductivity but also on the NO concentration which is regulated by the regional WSS as well. To our knowledge this is the first study that models LDL transport in a realistic 3D reconstructed coronary artery, taking into consideration the endothelial function.

In normal endothelium, LDL penetrates the arterial wall and its concentration is regulated, by exchanging the LDL molecules from the lumen to the wall and vice versa. In order to assess the impact that the endothelial function has on LDL accumulation we compared the LDL penetration in normal endothelium (assuming that its transport is based only on the WSS dependent hydraulic conductivity) to the LDL penetration in case of endothelial dysfunction. Fig. 5(a) illustrates the LDL concentration in normal endothelial function and 5(b) the difference of the LDL concentration between normal and endothelial dysfunction. It is obvious that the most significant differences appear at the regions with the maximum penetrations. More specifically, the calculated increase in LDL concentration is up to 46% (maximum LDL concentration: 6 mol/m^3 vs. 4.12mol/ m^3) in the model that takes into account endothelial dysfunction. This finding highlights the additional effect that the endothelium may have on LDL accumulation.

The proposed approach has several limitations which must be addressed in the future. First, NO production is more complex and does not depend only on the WSS. Accordingly Eq. 7 cannot describe accurately its concentration. In addition we assumed that there is a linear inverse relation between endothelial permeability and local NO concentration ignoring the role of other factors such as hypertension, vessel wall trauma or local inflammation. Though this

study has significant limitations it provides valuable quantitative information about the effect that the endothelial dysfunction may have on the atherosclerotic process.

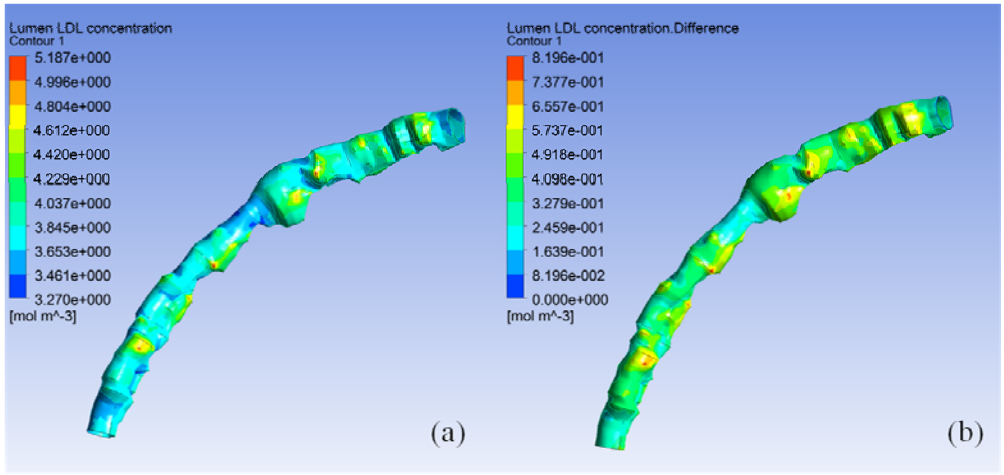

Fig. 5. (a) LDL concentration at normal endothelium, and (b) difference in LDL concentration between the case of endothelial dysfunction and the normal case.

The proposed model has several limitations which must be considered in the future. First, NO production is made by a complex network of chemical reactions. In our approach, the NO production is based only on the main chemical reaction (Eq. 7). Another limitation is that O_2 is not constant in the arteries but it is transferred by blood. Finally, endothelial dysfunction and abnormal concentrations of NO is found to be related with hypertension. Thus, the deformation of the arterial wall when the blood pressure increases must be taken into account.

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Извод

Моделирање акумулације LDL у случају оштећења ендотелних ћелија

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Резиме

Ендотелна ћелија је одговорна за нормалну хомеостазу крвних судова регулисањем неколико биолошких и хемијских механизама и одговора. Данас је евидентно да је ендотелно оштећење повезано са повећаним ризиком еволуције плака. Циљ овог рада је моделирање LDL транспорта и акумулације у реалним геометријама коронарних артерија у случају оштећења ћелија ендотела. За симулацију протока крви и LDL транспорта су коришћене Навије-Стоксова и конвективно-дифузиона једначине. Наш модел претпоставља зависност смичућег напона од хидрауличне проводљивости, али и повећане пропустљивости ћелије ендотела у случају ендотелне дисфункције. За ову сврху, производња ендотелних азотног оксида синтезе (eNOS) се користи за израчунавање синтезе азот оксида (NO). Прецизније, моделиране су хемијске реакције стварања NO и израчуната је концентрација која узима у обзир утицај на ендотелну пропустљивост. Добијени резултати показују да ендотелне дисфункције и NO концентрација имају додатни утицај на LDL акумулацију. Мале концентрације NO, повећавају пропустљивост ћелија ендотела што даје повећане наслаге LDL молекула у артеријском зиду. Добијени резултати показују да ниво акумулације LDL расте до 46% у случају оштећења ендотела, што потврђује улогу оштећења ендотелних ћелија на еволуцију плака.

Кључне речи: LDL, акумулација, моделирање, оштећење ендотелних ћелија

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