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3D Numerical Simulation of Coronary Blood Flow and its Effect on Endothelial Cell Activation

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Abstract— The goal of the present study was to develop a physiologically realistic 3D computational fluid dynamics (CFD) model of the left coronary artery under normal and disease conditions to estimate blood flow induced shear stress, and then use the computed shear stress to stimulate vascular endothelial cells *in vitro*. A 3D geometry of the left coronary artery was built in ProE and the CFD analysis of the flow field was carried out in Fluent (v 6.23) under normal, 30%, 60% and 80% stenosis conditions. The transient blood flow velocity and shear stress were solved using a *k*-ω turbulence model. 3 typical shear stresses at normal, 80% stenosis and recirculation zone levels were applied to vascular endothelial cells in a cone and plate shearing device. Endothelial cell activation and injury induced by shear stress were measured by cell surface ICAM-1 and tissue factor expression, using fluorescence microscopy. Results demonstrated that oscillatory low shear stress present in the recirculation zones can significantly activate endothelial cells by enhancing ICAM-1 expression; while elevated shear stress at stenosis can induce endothelial cell damage by enhancing tissue factor expression. This study demonstrated that the combination of CFD and *in vitro* studies provided an efficient way to investigate the mechanism of blood flow induced mechanical stress on cardiovascular disease development *in vivo*.

I. INTRODUCTION

Vascular endothelial cells (EC) play important roles in thrombosis, inflammation and atherosclerosis. Their activities are closely related to blood flow induced mechanical stresses. It is therefore critical to understand how the flow features and the stress field can affect the functions of vascular EC, as well as disease development. However, blood flow is time dependent, varies as the vasculature geometry changes and turbulence may develop at certain locations. Therefore, experimental measurements or estimations of shear stress applied to vascular wall EC could be problematic. Numerical simulation provides an alternative way to obtain detailed flow patterns and shear stress distribution. Building a realistic numerical model to calculate the stress applied to vascular EC *in vivo*, and realizing it precisely *in vitro,* is a helpful approach to study the responses of EC to complicated stress conditions.

Coronary artery disease is the most common heart disease and the leading cause of death in the United States. It is well established that due to the complex anatomical geometry, altered shear stress induced by coronary blood flow, especially under disease conditions (stenosis), may significantly affect the normal functions and activities of endothelial cells and lead to disease initiation and progression[1]. The aim of the present study was to develop a 3D numerical model of the left coronary artery with a realistic geometry under normal and stenosis conditions, compute wall shear stress distribution and apply the estimated wall shear stress to endothelial cells *in vitro* and examine their responses.

II. MATERIALS AND METHODS

A. CFD analysis of flow field and shear stress distribution in the left coronary artery

Pro/Engineering (Wildfire 4.0) and GAMBIT software package (v 2.4.6, ANSYS Inc.) were used to generate 3D geometries and meshes of the left coronary artery (Figure 1). The CFD analysis was conducted in Fluent (v 6.3.26, ANSYS Inc.). The dimensions of the 3D left coronary artery model are shown in Table 1[2,3]. The flow rate ratio between the two daughter vessels is 1:1. Disease conditions (stenosis) were modeled in the left anterior descending artery (circled area in Figure 1) by adding a restriction in the flow domain. The lumen narrowing (center point of stenosis throat) occurred 8 mm downstream the bifurcation. Three stenosis conditions – 30%, 60% and 80% -- were modeled in the present study.

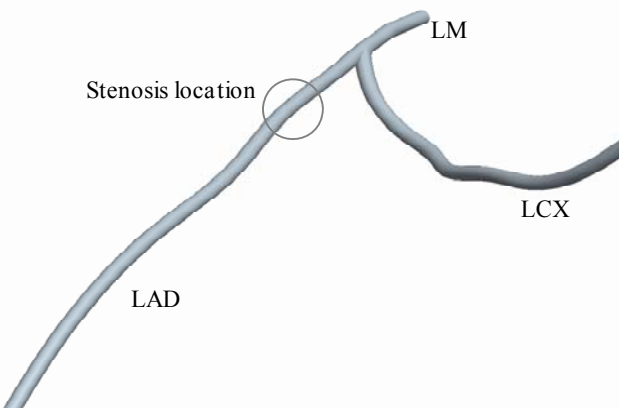

Figure 1. 3D model of the left coronary artery under normal condition, showing the three main branches: left main artery (LM), left anterior descending artery (LAD) and left circumflex artery (LCX). The circled area is the region of interest, where stenosis is located in disease models.

Table1. Dimensions of left main (LM) coronary artery, left anterior descending artery (LAD) and left circumflex artery (LCX) used in 3D CFD models.

	Parameters	3D (mm)
LM	Inlet Diameter	4.5
	Length	11
LAD	Inlet Diameter	3.8
	Length	118
	Outlet Diameter	1.9
LCX	Inlet Diameter	3.7
	Length	76.8
	Outlet Diameter	1.7
Bifurcation Angle		74.83°

When a blood vessel becomes significantly narrowed (stenosis), blood flow can be severely restricted and turbulence and recirculation zones may develop. The turbulent stresses may easily exceed their laminar counterparts by an order of magnitude. Thus, the laminar and Newtonian model is not the best approach. To solve the sharply varying flow variables in near-wall regions, especially in the viscous sub-layer of the turbulent field (where Reynolds number of the flow is relatively low), the Unsteady Reynolds Averaged Navier-Stoke (URANS) equations using the innovative Wilcox k-ω model[4] was primarily used to solve globally low-Re internal flows (intermittent turbulent flows in the transitional range). The Wilcox's k-ω model is able to account for sudden changes in main-strain rate and predict the variation of turbulence variables through the viscous sub-layer all the way to the vessel wall. The inlet velocity (fully developed) waveform is shown in Figure 2, with the flow velocity ranging between 0.2 and 30 cm/sec with a period of 0.84 sec. Blood was modeled as a non-Newtonian viscoelastic fluid, with a density of 1.2 g/ml and a yield stress of 0.1/s. Shear stress along the vascular wall under normal and stenosis conditions was calculated using velocity gradient and local effective turbulent viscosity.

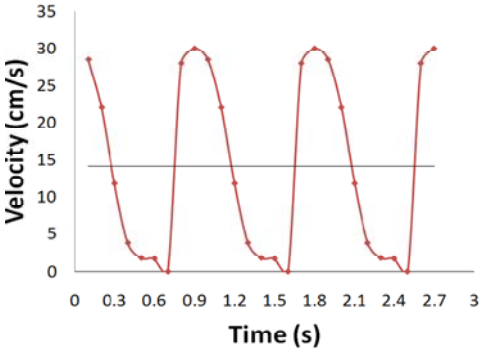

Figure 2. Inlet waveform of the coronary blood flow (3 cardiac cycles).

B. Endothelial cell activation and injury induced by shear stress

Human bone marrow microvascular endothelial cells (BMEC) were obtained from Dr. Barbette Weksler at Department of Hematology and Oncology, Weill Medical College of Cornell University (New York, NY), and cultured in Dulbecco's minimal essential media (DMEM), supplemented with 5% fetal bovine serum (FBS), 10 mM HEPES, and 1:100 Penicillin/Streptomycin. BMEC were grown on 0.2% gelatin and used between passage 14 and 28. Cells were grown to confluence on a 6-well cell culture plate before use. Transient wall shear stresses at our point of interest (circled area in LAD, shown in Figure 1) estimated from CFD simulation were imported into a dynamic cone and plate shearing device and applied to BMEC for 30 min at 37°C, in the presence of 500 µl culture media with 0.5% FBS. After shearing, culture media was removed and the EC monolayer was washed (3x) with TBS (Tris buffered saline, Ph7.4) and then fixed with 0.5% glutaraldehyde for 15 minutes at 37 °C. After neutralization in 100 mM glycine - 0.1% BSA for 30 minutes, and washing (3x) with TBS, EC activation was measured using murine monoclonal anti-human ICAM-1 antibody (1µg/ml, Ancell Corporation, San Diego, CA). After incubation (1 hour, 37 °C) and washing (3x) with TBS, primary antibody binding was detected using Alex Fluor 488 conjugated goat anti mouse secondary antibody (1:100, Molecular Probes) for 30 min at room temperature (RT). To examine shear stress induced endothelial injury, EC surface tissue factor (TF) expression was assessed in a similar manner by using monoclonal murine anti human TF antibody (10µg/ml , Abcam) (1 hr, RT). After washing (3x) in TBS, the reaction was examined using fluorescence microscopy (Nikon TE 2000U).

III. Results

Velocity vectors and specific cross sections near the stenosis in 3D models are depicted in Figure 3. Different from normal blood flow, flow in restricted vessels accelerated at the stenosis throat, with noticeable

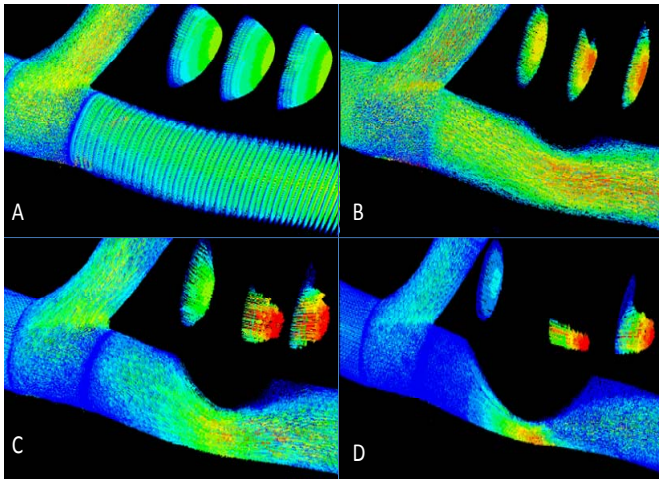

Figure 3. Velocity vectors in the flow field and at specific cross sections near stenosis. A) Normal; B) 30% stenosis; C) 60% stenosis; D) 80% steenosis.

recirculation zones developed behind the stenosis. As stenosis severity increases, flow accelerates more and recirculation zones grow bigger.

Shear stress in the flow field changes with time, with the maximum stress developed around 800 ms after systole. Shear stress distribution 800 ms after systole on the flow cross sections 8 mm (stenosis throat for stenosis conditions) downstream of the bifurcation is shown in Figure 4. Maximum shear stress occurs on the vessel wall, with the stenosed wall shear stress being much higher (25, 32 and 142 dynes/cm^2 respectively for 30%, 60% and 80% stenosis) than that under normal conditions (14 dynes/cm^2).

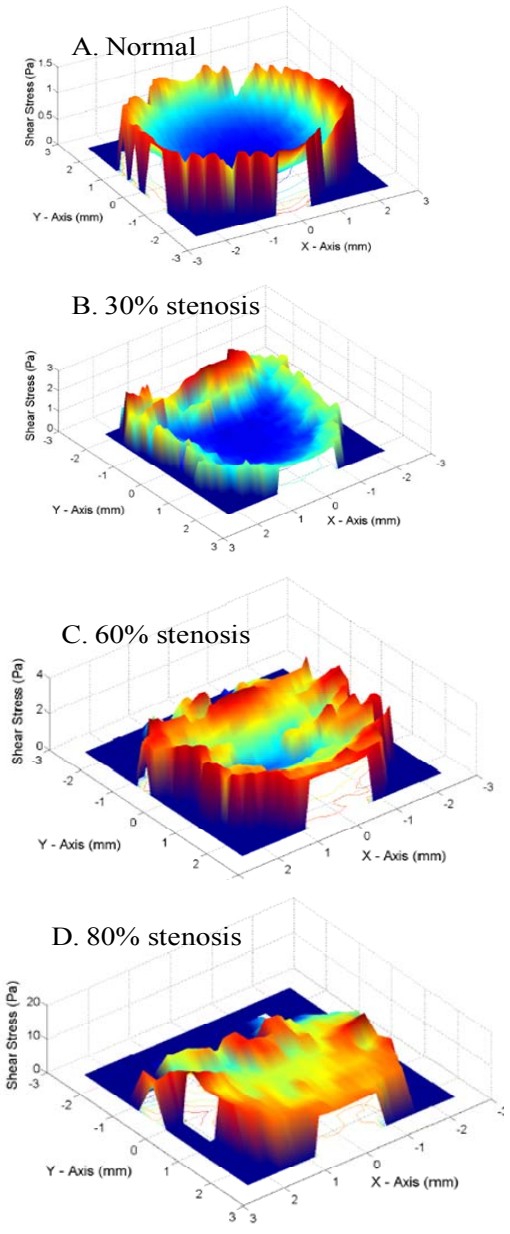

Figure 4. Wall shear stress distribution 8 mm downstream the bifurcation (where stenosis throat is located), 800 ms after peak systole.

However, the wall shear stress in the recirculation zones (starting from 12 mm downstream bifurcation, Figure 5) is much lower (< 10 dynes/cm^2). In one cardiac cycle (shear stress distribution at other time points are not shown), shear stress under the normal condition varies between 1 and 14 dynes/cm^2. With a stenosis present, blood flow induced shear stress varies significantly (2-25 dynes/cm^2 for 30% stenosis, 2- 32 dynes/cm^2 for 60% stenosis and 2-142 dynes/cm^2 for 80% stenosis), while that in the recirculation zone remains

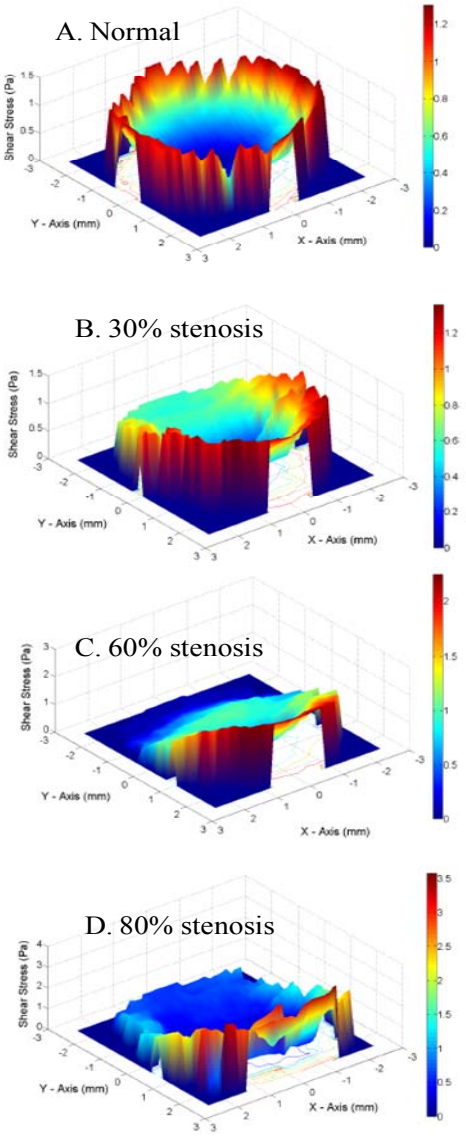

Figure 5. Wall shear stress distribution 12 mm downstream the bifurcation (where the recirculation zone starts), 800 ms after peak systole.

below 10 dynes/cm^2 with little variation at all time. Also, due to the stenosis, shed vortices start to generate downstream of the stenosis throat, which induced a decrease and fluctuation in wall shear stress.

Wall shear stress obtained from CFD was used to stimulate endothelial cells in the cone and platelet shearing device. The 3 different wall shear stress waveforms used in the present study are shown in Figure 6, representing wall shear stress on normal vessel wall, stenosed vessel wall (80% condition, near stenosis throat) and in recirculation zones. The result (typical images are shown in Figure 7) demonstrated that pathological shear stresses (recirculation zone and stenosis) can significantly enhance both tissue factor (TF) and ICAM-1 expression on endothelial cells. Oscillatory low wall shear stress found in the recirculation zones has more effect on EC surface ICAM-1 expression (Figure 7A-C). Compared to elevated shear stress, the low oscillatory shear stress has a higher potential to induce ICAM-1 expression, suggesting its role in EC activation. While elevated wall shear stress found near the stenosis throat has a greater effect on tissue factor expression (Figure 7D-F), indicating its role in inducing tissue damage and promoting thrombosis.

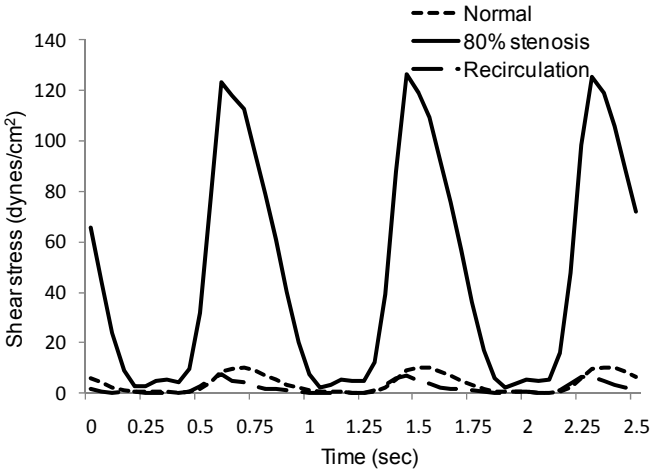

Figure 6. One set of waveforms obtained from numerical simulation under 80% stenosis condition. These three waveforms were applied to bone marrow microvascular endothelial cells *in vitro*.

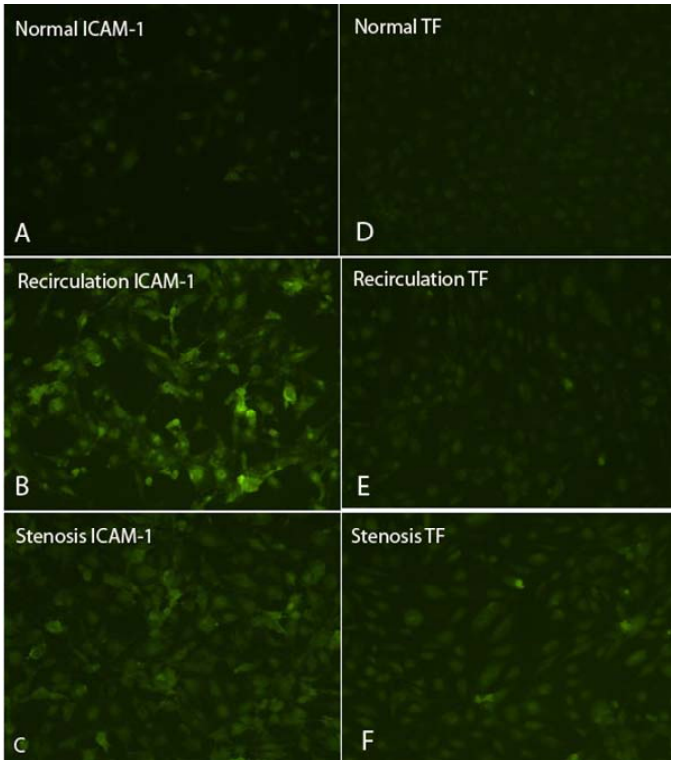

Figure 7. Endothelial cell surface ICAM-1 and tissue factor expression after they were exposed to pulsatile shear stress at normal, recirculation zone and stenosis (80%) level.

IV. CONCLUSION

Numerical simulation has become an efficient tool to study flow field and blood flow induced shear stress in the cardiovascular system. However, very few numerical models were successful to guide *in vitro* experiments. In this study, a realistic 3D computational fluid dynamics model was developed to estimate transient wall shear stress conditions in the left coronary artery. Information obtained from this model was used to direct *in vitro* experiments, to mimic *in vivo* conditions. Results obtained from this study provided a new role of numerical simulation in biomedical research, and can help us better understand the mechanism of endothelial activation and vascular wall damage accumulation and propagation. Furthermore, the *in vitro* studies can serve to improve the accuracy of the numerical model and help to make it a powerful tool to predict disease development.

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