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Research Article

Concentration Polarization of High-Density Lipoprotein and Its Relation with Shear Stress in an In Vitro Model

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The purpose of this study was to determine the concentration polarization of high-density lipoprotein (HDL) at the surface of the carotid artery under conditions of steady flow and to establish its relationship with shear stress using an in vitro vascular simulation model of carotid bifurcation. Shear stress, HDL concentration at the surface, and the ratio of HDL concentration at the surface to concentration in bulk flow were measured at different locations within the model under high-speed (1.451 m/s) and low-speed (0.559 m/s) flow. HDL showed concentration polarization at the surface of the carotid artery model, particularly in the internal carotid artery sinus. With decreasing flow velocity, the shear stress at the surface also decreased, and HDL concentration polarization increased. The concentration polarization of HDL was negatively and strongly correlated with shear stress at both low- ($r = -0.872$, $P < .001$) and high-speed flow ($r = -0.592$, $P = .0018$).

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1. Introduction

Vascular events induced by atherosclerosis are the leading cause of death in developed countries. While the pathogenesis of atherosclerosis involves a series of events, the end result is the formation of an atherosclerotic plaque [1]. The preliminary stage of atherosclerosis is the presence of lipids in the vascular wall caused by subendothelial retention of lipoproteins [2]. High plasma concentrations of LDL-c (low-density lipoprotein cholesterol) and low plasma concentrations of HDL-c (high-density lipoprotein cholesterol) are each independent risk factors for the development of atherosclerosis [3, 4]. LDL has been shown to be the major carrier of cholesterol and the main atherogenic lipoprotein for atherosclerosis. Its oxidized form (Ox-LDL) binds to endothelial cells and leads to apoptosis [5]. HDL exerts a well-documented antiatherogenic effect [6], and the mechanisms underlying the antiatherogenic effect of HDL include reverse cholesterol transport [7–9], anti-LDL oxidation [10, 11], and the protection of endothelial cells [12–15].

However, low HDL-c (high-density lipoprotein cholesterol) plasma concentrations may directly promote atherogenesis, particularly when LDL-c (low-density lipoprotein cholesterol) is elevated. In areas with low shear stress, increase of NADPH oxidase expression and defect of oxygen transmission enhance the production of reactive oxygen species ($O_2^{-\cdot}$, H_2O_2, etc.), accelerate the oxidation of LDL, and produce Ox-LDL [16, 17]. Ox-LDL can induce MCP-1 expression and result in adhesion and migration of the monocyte [18]; it also can combine with its specific LOX-1 receptor to decrease the expression of Bcl-2 and c-IAP-1 and induce apoptosis [19]. Therefore, concentration polarization of LDL provides sufficient substrates for the pathogenic ox-LDL, together with the deranged low shear stress environment, makes the endothelial cells in the area with low shear stress more vulnerable to the damage, and makes the occurrence of atherosclerosis easier.

In addition to concentrations of LDL and HDL in systemic circulation, some physical and fluid mechanical factors play an important role in the development of atherosclerotic plaques [20, 21]. Concentration of LDL and HDL at the

surface of the vascular wall rather than in the circulation (bulk flow) plays an important role in the process of lipid infiltration and atherogenesis. The concentrations of LDL at the surface of the arteries are reportedly higher than LDL concentration in the bulk flow. This phenomenon is referred to as "lipid concentration polarization" [22]. Concentration polarization of lipoproteins is affected by three major factors: wall shear stress, filtration velocity of water at the vessel wall, and the distance from the entrance of the artery [21, 23]. Shear stress, the internal force per unit area of an artery that is formed to resist external factors [20, 21, 24], is low in vessels where flow is disturbed and/or slow. The surface concentration of LDL is locally elevated in regions of low flow and low shear stress, which provides a favorable condition for the formation of atherosclerotic plaques [22].

In the last 10 years, more and more researchers have been paying close attention to the effect of hemodynamics on material transfer and the interaction of blood constituents with the vessel wall. This interest has generated a theory that the retention time of this noxious substance and its deposition on the vessel wall are correlated with atherosclerosis and thrombosis. In addition, concentration polarization is thought to affect both the retention time of LDL and its deposition on the vessel wall [25].

To date, research on lipid concentration polarization has focused on LDL. But studies on concentration polarization of HDL are also essential for understanding the development and localization of atherosclerosis. Therefore, we studied the intraarterial localization of HDL during low-flow and high-flow conditions, using a carotid bifurcation vascular model which designed to mimic the configuration of the human carotid artery. The purpose of this study was twofold: to determine if concentration polarization exists for HDL and to determine whether concentration polarization is related to shear stress. The results of such a study would provide additional information regarding the concentration polarization and polarization location of HDL that might be helpful in understanding the pathogenesis of atherosclerosis.

2. Materials and Methods

2.1. Experimental Model. Semipermeable poly tetra fluor ethylene (PTFE) was employed to create the carotid bifurcation model. The inner diameter of each region of a human carotid artery was measured by Doppler ultrasound and computerized tomography angiography. Because a previous model at 1:1 resulted in uneven endothelialization and other technical difficulties, these measurements were amplified at a rate of 1:1.5 (Table 1) experimentally to create a bifurcation model of the carotid artery that was subsequently coated with endothelial cells on the inner wall of the model to induce the endothelialization of the inner wall of the model as shown in Figure 1 [26].

As shown in Figure 2(a), the model included the common carotid artery (CCA), external carotid artery (ECA), internal carotid artery (ICA), and internal carotid artery sinus (ICAS). The low shear stress core region and its margins were marked on the model. The low shear stress core region was marked as point 5, and the anterior and posterior margins were marked as points 3 and 4 (Figure 2(b)). The measuring points of the inner diameter of the CCA and the inner diameter of the ICA were used as control points and marked as points 1 and 2 (Figure 2(b)). In addition, the final model is provided in the schematic diagram in Figure 3(a), and the schematic diagram of the experimental system was shown in Figure 3(b).

Table 1: The inner diameter of carotid artery measured by Doppler ultrasound and computerized tomography angiography and the model measurements obtained by amplifying the actual values by 1.5.

Measuring position	Measuring size (mm)	Model size (mm)
Common Carotid Artery (CCA)	6.5	9.8
Internal Carotid Artery (ICA)	4.9	7.4
External Carotid Artery (ECA)	4.2	6.3
Internal Carotid Artery Sinus (ICAS)	7.8	11.7

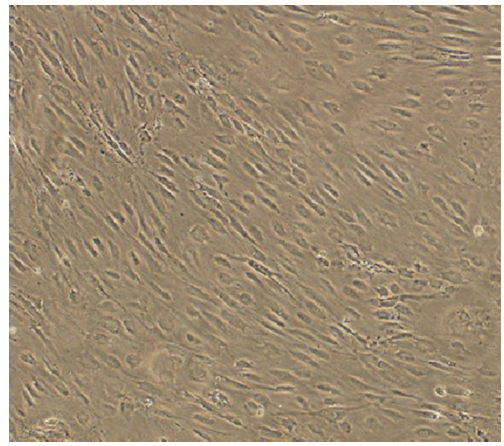


Figure 1: Observation of cell growth and cell arrangement with a converted microscope after implanting the inner surface of the carotid bifurcation model (magnification × 100).

2.2. Numerical Simulation Methodology. If the circulating liquid is taken as an incompressible Newtonian fluid, then the flow is a steady flow. If the volume power, heat exchange, and other physical and chemical factors are not considered, then the equations provided in the footnote can be employed. The first formula is a continuity equation, and the second formula is an equation of motion where u_i is the velocity of flow field, P is the fluid pressure, ρ is the fluid density, and μ is the fluid viscosity. Because the basic equations mentioned above are intensive nonlinear equations, the Finite Volume Method (FVM) was employed, because it is the most commonly used numerical method for resolving this type of mathematical problem at present [27].

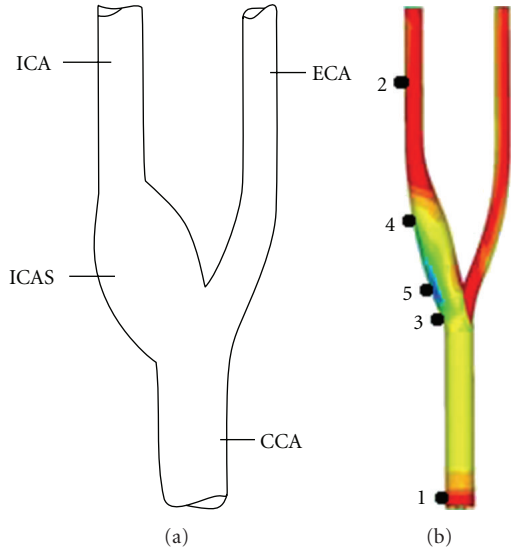


Figure 2: (a) Pictoral illustration of the carotid bifurcation vascular model. CCA: common carotid artery, ICA: internal carotid artery, ECA: external carotid artery, ICAS: internal carotid artery sinus. (b) The distribution of the shear stress in the carotid bifurcation vascular model. Color changes show the degree of shear stress. Blue represents the lowest shear stress while red is the highest. 1 and 2: control locations of CCA and ICA; 3 and 4: anterior and posterior edges of the low shear stress region, 5: core region of the low shear stress area.

The FLUENT software is hydrodynamic calculating software based on the FVM, and it is the CFD software application most widely utilized:

$$\frac{\partial u_j}{\partial x_j} = 0,$$
$$\rho \frac{\partial u_j u_i}{\partial x_j} = -\frac{\partial P}{\partial x_i} + \mu \frac{\partial}{\partial x_j}\left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i}\right). \quad (1)$$

2.3. Hydrodynamic Parameters of Blood Flow. The parameters of the blood flow in the model were controlled by the particle image velocimetry (PIV) using a type PIV-400-10 (TSI Company, Shoreview, MN, USA). The average flow velocity of ICA was set at 0.559 m/s, which is the average flow velocity within the ICA measured in the human body at 150 mmHg blood pressure. PIV was also used to record the parameters of the blood flow in the core low shear stress region and at its margins.

A solution prepared with 7.5% glycerol with a viscosity of 0.782 mPa.s and a density of 1.005×10^3 kg/m^3 (to prerun and stabilize the equipment) was measured by the Low Shear 30 (CONTRAVES LOW SHEAR 30 ISCOMETER, Swiss) and used in the PIV measurements as described previously [24]. This viscosity was chosen because it was the same viscosity as the M199 culture medium used for the endothelial cells. Blood flow parameters determined were flow rate (mL/s) and velocity of the circulation liquid through the model (flow velocity, m/s).

Table 2: Hydrodynamic parameters for low-speed and high-speed flow groups.

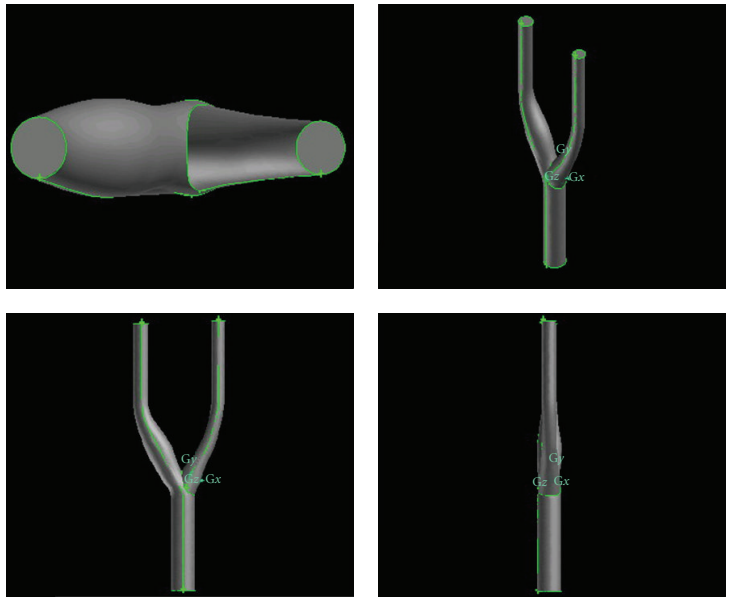
Group	Flow Rate (mL/s)	Flow Velocity (m/s)
Low-speed	60.62	0.559
High-speed	155.93	1.451

2.4. Separation of HDL and LDL. Human plasma lipoproteins were collected and separated using the one-time density gradient ultracentrifugation method described by Zhang and Liu [28]. HDL and LDL bands were collected from centrifuged samples using a long syringe needle and were dialyzed in a buffer containing 0.02 mol/LTris-HCl, 0.85% NaCl, 0.01% EDTA, and 0.01% NaN_3 at pH 7.6. Dialysis was performed at 4°C in the dark for 6 hours each time and completely repeated 4 times in order to remove sodium bromide. Collected lipoproteins were stored at 4°C following filtration (storage and dialysis were performed under a nitrogen atmosphere to avoid oxidation). Figure 4 shows the high purity of the isolated LDLs and HDLs. In total, 100 mL of circulation liquid was prepared for the experiments, including 80 mL of M199 medium and 20 mL of separated human plasma lipoproteins (i.e., 10 mL LDL and 10 mL HDL). Lipoprotein concentrations were determined with an OLYMPUS automatic biochemical analyzer (OLYMPUS automatic biochemical analyzer AU2700, Japan). The concentrations of LDL and HDL in the bulk (C_0) were 0.575 mmol/L and 0.242 mmol/L, respectively.

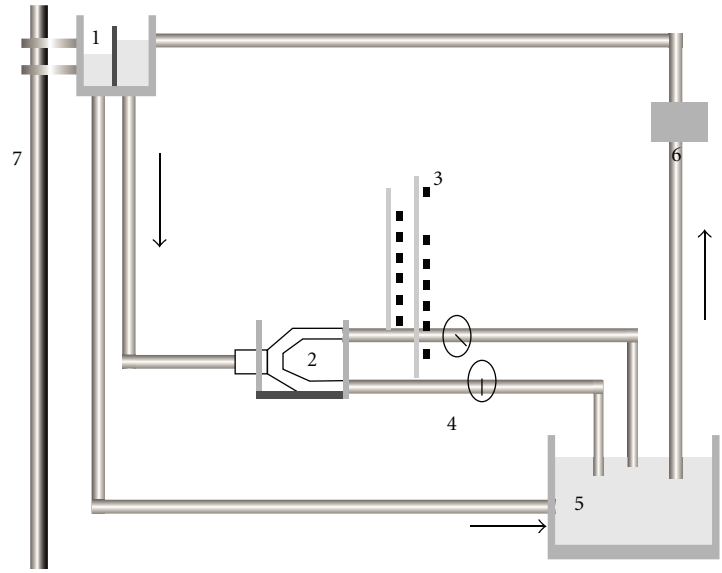
2.5. Experimental Procedure. HDL concentration polarization was measured under two different ICA flow velocities at 5 different locations in the model. The low-speed group of measurements had an average ICA flow velocity of 0.559 m/s (the average flow velocity of ICA in human body under 150 mmHg blood pressure) while the high-speed measurement group had an average ICA flow velocity of 1.451 m/s (the peak flow velocity of ICA in human body under 90 mmHg blood pressure). Hydrodynamic parameters (i.e., blood flow in the low- and high-speed groups) were measured and are summarized in Table 2.

After allowing the model to stabilize for 30 minutes, 50 μL samples were sequentially collected from each of the 5 locations, and 5 samples were collected consecutively from each location. The samples were collected 15 minutes apart to ensure that samples were collected at a constant flow. The collected samples were individually placed in polyethylene tubes and stored protected from light in brown bottles at 4°C. Lipoprotein concentrations were measured within 4 hours of collection. The ratio of the concentration of HDL at the surface (C_S) to the concentration in bulk (C_0) was used as an index for concentration polarization of HDL. Polarization of HDL was considered to have occurred if the ratio was greater than 1.000.

2.6. Statistical Analysis. Study variables were presented using descriptive statistics (mean, median, and range). Differences in variables between the five locations in the model were



(a)



(b)

Figure 3: (a) Schematic diagram of the numerical simulation model (four different viewsor angles). (b) The schematic diagram of the experiment recirculate system. Indicating points are (1) upstream reservoir, (2) carotid bifurcation vascular model, (3) manometer, (4) flow meter, (5) downstream reservoir, (6) centrifugal pump, and (7) slide pole. Working process of flow chamber system: according to the requirement of the experiment, the water flow system was controlled by the water levels of the upstream and downstream chambers to maintain the stability of flow rate for a long time, and 12 L measuring cylinder was applied in the current experiment to measure the flow rate at the spillway of the downstream. The water level of the chamber was regulated with the moving plate to stabilize the difference of water level between the upstream and downstream. A grid plate was used to separate the upstream chamber to maintain the stable water flow in the chamber. The horizontal status of the experimental platform was measured and regulated with the leveling instrument. After flowing out from the downstream chamber, the circulating liquid flew into the upstream chamber again with the help of the centrifugal pump and took part in the circulation.

evaluated by Kruskall-Wallis tests. The Mann-Whitney U test was used to calculate differences between locations at the same flow speed or between two speeds at the same location. Pearson correlation analysis was performed to determine the correlation between shear stress and concentration polarization of HDL. All statistics analyses were performed in SAS software version 9.1.3 (SAS Institute, Cary, NC, USA). All tests were two-sided and 0.05 was used as the significance level. For multiple comparisons, adjusted α was defined as α/number of comparisons.

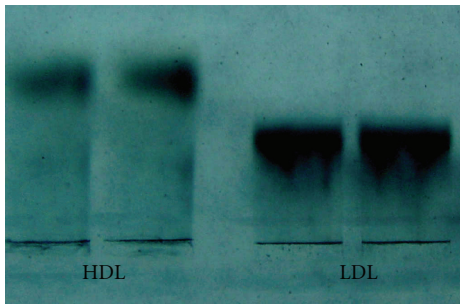


Figure 4: Purity of HDL and LDL banding patterns in gel electrophoresis after density gradient ultracentrifugation.

3. Results

The shear stress was the highest at location 1 under high-speed flow (58.33 dyne/cm^2), and was the lowest at location 5 under low-speed flow (0.69 dyne/cm^2) in this in vitro model of the carotid bifurcation. The shear stress, surface concentration of HDL (C_S), and C_S/C_0 ratio at the two speeds of flow and five locations in experimental model are summarized in Table 3. At the low-speed flow, the C_S of HDL was the highest at location 5 and the lowest at location 2 ($P < .001$). Similarly, Cs was also the highest at location 5 and the lowest at location 2 at the high-speed flow rate ($P < .01$). For locations 3, 4, and 5, HDL surface concentrations were significantly higher under low-speed flow compared with high-speed flow ($P < .01$). A similar pattern was observed for the ratio of C_S/C_0: it was the highest at location 5 and the lowest at location 1 for both low- and high-speed flow ($P < .001$ and $P = .049$, resp.). At locations 3, 4, and 5 where shear stress was low, the C_S/C_0 ratio was significantly higher at low-speed flow compared with high-speed flow ($P < .01$).

Concentration polarization of HDL (ratio of $C_S/C_0 > 1.000$) was present at all locations, except location 1 (control location of CCA) under low-speed flow. The data indicate that a reverse association exists between shear stress and both C_S and the ratio of C_S/C_0 in that the lower the shear stress, the higher the C_S and ratio of C_S/C_0 (Figure 5). In the correlation analysis, the ratio of C_S/C_0 was negatively and strongly correlated with shear stress at both low- and high-speed flow (correlation coefficient $r = -0.872$, $P < .001$; $r = -0.592$, $P = .0018$, resp.).

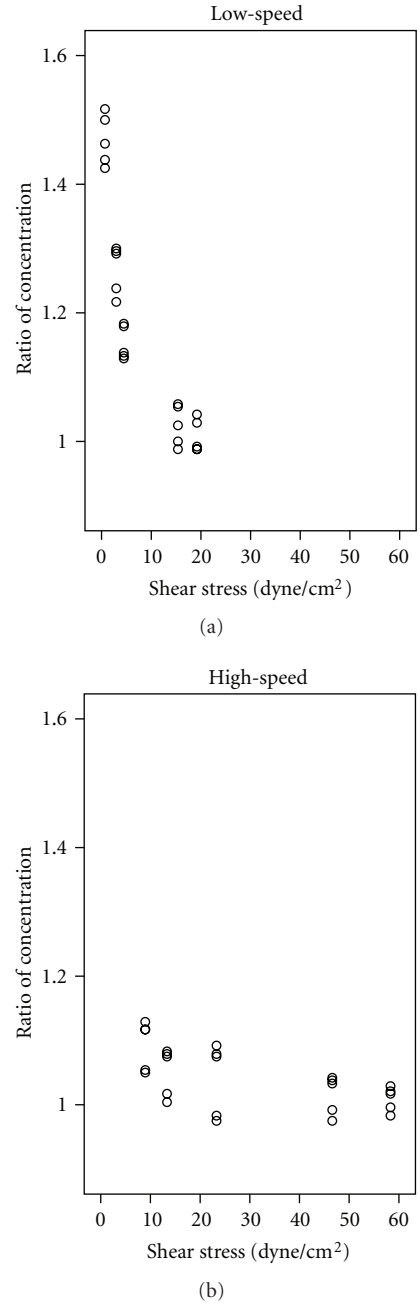


Figure 5: Scatter diagrams of shear stress and ratio of C_S/C_0 under low- and high-speed flow and correlation coefficients (r).

4. Discussion

In the present study, an in vitro carotid artery bifurcation model was created and employed to explore the concentration polarization of HDL to further elucidate the pathogenesis of atherosclerosis. The results revealed that HDL shows concentration polarization at the surface of the carotid artery, particularly in the internal carotid artery sinus, a low shear stress region. In addition, the concentration polarization of HDL was found to be negatively and strongly correlated with shear stress for both low- and high-speed flow velocities.

Clinical studies and human autopsy reports indicate that atherosclerosis often occurs in the carotid artery, coronary artery, abdominal aorta, and peripheral arteries, that is, locations where the vascular geometric shape of the vessel changes sharply due to the formation of either vascular branches, abrupt changes in direction (bends), or vascular stenosis [29]. In our model, an obvious low shear stress region was identified at the surface of the internal carotid artery sinus. When the model's flow velocity decreased from 1.451 m/s to 0.559 m/s, the absolute value of the shear stress of this low shear stress region decreased from 8.96 dyne/cm^2

Table 3: Experimental variables in five locations of model where samples were taken and analyzed for low-speed and high-speed flow.

		Location 1	Location 2	Location 3	Location 4	Location 5	P-value[b] e[a]
Shear stress (dyne/cm^2)[a]	Low-speed	19.21	15.37	4.48	2.97	0.69	—
	High-speed	58.33	46.61	23.29	13.34	8.96	—
C_S for HDL (mmol/L)[a]	Low-speed	0.238 (0.237–0.250)	0.246 (0.237–0.254)	0.273[d] (0.271–0.284)	0.310[d,e] (0.292–0.312)	0.351[d,e,f] (0.342–0.364)	<.001
	High-speed	0.244 (0.236–0.247)	0.248 (0.234–0.250)	0.258 (0.234–0.262)	0.258 (0.241–0.260)	0.268[d] (0.252–0.271)	<.01
	P-value[c]	—	.69	<.01	<.01	<.01	
C_S/C_0 *ratio* for HDL[a]	Low-speed	0.992 (0.988–1.042)	1.025 (0.988–1.058)	1.138[d] (1.129–1.183)	1.292[d,e] (1.217–1.300)	1.463[d,e,f] (1.425–1.517)	<.001
	High-speed	1.017 (0.983–1.029)	1.033 (0.975–1.042)	1.075 (0.975–1.092)	1.075 (1.004–1.083)	1.117[d] (0.050–1.129)	.049
	P-value[c]	—	.69	<.01	<.01	<.01	

C_S: concentration at the surface, C_S/C_0: ratio of the concentration at the surface (C_S) to the concentration in the bulk circulation (C_0).
[a]Continuous variables were presented as median (range) except for shear stress. Shear stress was constant. [b]Kruskall-Wallis test was used to test the differences among locations, $P < .05$ was the level of statistical significance, and Mann-Whitney U test with adjusted α was used to test the differences between each two locations (adjusted $\alpha = \alpha/6 = 0.0083$). [c]Mann-Whitney U test was used to test the difference between speeds. $P < .05$ was the level of statistical significance. [d]$P < .0083$ versus location 2. [e]$P < .0083$ versus location 3. [f]$P < .0083$ versus location 4.

to 0.69 dyne/cm^2. Since the internal carotid artery sinus is an anatomic location with a high incidence of atherosclerosis, additional in-depth studies are clearly warranted.

Fatouraee et al. [20], examined canine straight carotid arteries and found that under the conditions of steady and pulsatile flow, LDL concentrations at the surface were 7%–16% and 5%–14% higher than the bulk concentration, respectively. Our research group previously indicated that LDL showed concentration polarization at different flow velocities using an in vitro model [30]. In the present study, HDL concentrations at the surface at each sampling location (except control location 1 at low speed) were higher than those in the bulk flow, which indicates that HDL also has concentration polarization. In addition, it appears that at the same flow velocity, the extent of the low shear stress region at the surface of internal carotid artery sinus was relatively large with an obvious low shear stress core region. The degree of HDL concentration polarization at low shear stress region was much higher than that at the straight pipe part (control locations of CCA and ICA). At different flow velocities, the shear stress at the surface of internal carotid artery sinus had different degrees of changes, and the degree of HDL concentration polarization had a greater range of fluctuation. In fact, a negative correlation between shear stress and HDL concentration polarization was found, and the concentration polarization decreased with the increasing shear stress at both flow velocities.

The present study used a model of carotid artery bifurcation under steady flow as a platform that can simulate the internal carotid artery sinus and the mechanical characteristics of the local flow field. This model also included implanted endothelial cells in order to better mimic an in vivo artery and create a permeable model. In this model, when the flow velocity was low at 0.559 m/s, the low shear stress region at the surface of internal carotid artery sinus was larger, with an obvious low shear stress core region. More importantly, this model mimics the configuration of human carotid artery anatomy more faithfully than the previous carotid straight pipe model and straight pipe model with branches [31]. Thus, the model used in the present study can reflect the local hydrodynamic characteristics of internal carotid artery sinus more reliably in vitro.

The major limitation of this study was that it studied HDL only in normal concentration ranges. Further studies are needed to assess the concentration polarization character of HDL in abnormal concentration ranges as would be observed for most patients with atherosclerosis. Other limitations are that we did not measure the concentration polarizations of HDL and LDL in the same flow field, adjust the parameter of flow field (flow rate), and calculate the change of the difference between the concentration polarization of HDL and that of LDL in a same point along with the change of flow rate to further explain the interaction of the concentration polarization of HDL and that of LDL during the development of atherosclerosis. It is also noted that the present study is an in vitro experiment which cannot completely imitate the in vivo environment. The concentration polarization of HDL was proved under in vitro condition during the present study, and we hope in the future to construct an animal model in which the diameter of the common carotid artery will be changed by the surgical approach to produce various flow rates at the entrance for observing and verifying the concentration polarization of HDL in vivo.

Nonetheless, the present study has shown that concentration polarization exists in HDL and therefore is a potential risk factor in the process of atherosclerosis. In our future research, we plan to perform a comprehensive study of the degree of concentration polarization of both HDL and LDL under different flow velocities in the same or a similar model to explore the effect of concentration polarization of two different lipoproteins on the atherogenesis. This will

also enable us to investigate potential ways to upregulate concentration polarization of HDL in order to prevent or slow atherogenesis.

5. Conclusion

In summary, at the two different flow velocities employed in this in vitro study, concentration polarization of HDL was identified at the surface at various points in the carotid bifurcation model. In particular, concentration was obvious in the internal carotid artery sinus, it was inversely related with shear stress and flow velocity. This observation is important as it communicates the inaugural finding of concentration polarization involving HDL in the internal carotid artery sinus, which is an anatomic location associated with a high incidence of atherosclerosis. The findings of this suggest that LDL and HDL both contribute, perhaps interactively, in the development of atherosclerosis in low shear stress regions of the carotid bifurcation. Further studies based on the present findings are warranted.

Acknowledgments

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