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Endothelial Cells: The Living Sensors and Actuators

Tzung K. Hsiai, Michael Ing, Juliana Hwang, Adler Salazar

Biomedical Engineering and Cardiovascular Medicine, School of Engineering and Keck School of Medicine, University of Southern California, Email: Hsiai@usc.edu

Sung K. Cho, Pak Wang, Chih-Ming Ho

Mechanical and Aerospace Engineering, SEAS, UCLA, skc@ucla.edu

Mohamad Navab

Division of Cardiology, UCLA School of Medicine, mnavab@mednet.ucla.edu

Abstract

Cells function as an integral part of living tissues, constantly sensing and responding to biochemical and biomechanical stimuli. Endothelial cells (EC), in particular, are able to discriminate both the temporal and spatial components of shear stress, rending cell signaling, gene expression, and followed by protein formation. We embraced the MEMS technology to characterize real-time shear stress on EC. We provided the first *in vitro* evidence whereby a distinct pattern of cell-cell binding kinetics developed in parallel with dynamic gene expression in response to oscillatory shear stress.

Key words: Shear stress, endothelial cells, monocytes, gene expression

1. Introduction

Endothelial cells (EC) line the inner lumen of blood vessel, serving as an interface between blood and tissues. EC are able to discriminate the distinct characteristics of shear stress, unidirectional vs. reversal flow, and subsequently, expressing genes promoting or attenuating cardiac disease[1]. We fabricated MEMS shear stress sensors, which were comparable to an elongated endothelial cell, to link real-time shear stress with EC sensing, signaling, and subsequent gene expression and cell-cell interactions. By virtue of its small dimension (2 x 80 μ m) and resistive property (frequency responses > 100 Hz), the MEMS sensor enabled us to resolve the spatial and temporal resolution for biomedical research[2]. Simulating pulsatile flow profiles known to occur in the arterial circulation, we demonstrated the effects of shear stress induced EC signaling through gene expression, namely, mRNA of monocyte chemoattractant protein-1 (MCP-1), and subsequent EC binding to monocytes through the distinct trajectory patterns of cellcell interactions.

2. Methods

The operation of shear stress sensor has been described based on fully developed flow condition in which the rate of heat loss from a heated resistive element to the fluid-

flow is dependent on the boundary–layer velocity profile; thus, the shear stress[3]. A linear relation between V^2 and $\tau w^{1/3}$ can be derived as $V^2/R \propto \tau^{1/3}$:

$$V^2/R = (A_T + B_T \tau^{1/3}) \quad (1)$$

where A and B are the calibration constants. For our investigation, a constant temperature (CT) mode circuit was selected for its high frequency bandwidth crucial in oscillatory measurement, low over heat ratio and short response time4. The sensing resistance, R, is kept at constant by a feedback circuit using the Wheatstone bridge design. Since $P = V2/R$, V is the voltage across the sensor.

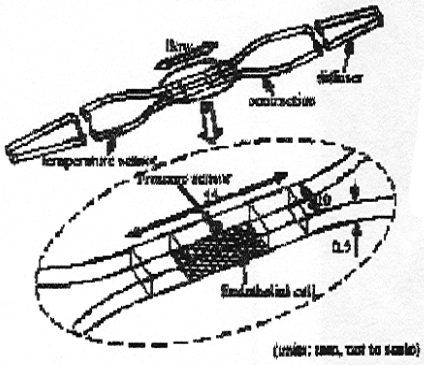


Fig. 1 Pulstile flow channel showing the details of testing chamber.

The flow system was designed to deliver welldefined flow profiles simulating pulsatile and oscillating flow conditions in arterial circulation (Fig.1). The theoretical formulation can be accessed on line at http://atvb.ahajournals.org[5].

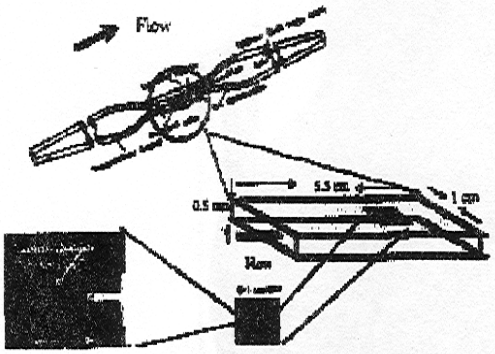


Fig. 2 The MEMS sensor chip was embedded in the upper wall while the EC monolayers were seeded on the lower wall.

By the symmetry of rectangular flow channel, we have embedded the sensor opposite to the EC monolayers seeded on the bottom of the parallel plate channel (Fig. 2). The sensor featured the polysilicon as the heating and sensing element at the center of a cavity diaphragm. The polysilicon, measured at 2 μ m wide, 0.5 μ m high, and 80 μ m long, were uniformly doped with Boron to a low sheet-resistance value of 50Ω/cm^2 with a typical resistance between 1.25 ~10 kΩ. at the room temperature.

Endothelial Cell Culture BAECs (Bovine aortic EC) between passages 5 and 9 were seeded on Vitrogen (Cohesion, Palo Alto, RC 0701) coated glass slides (1cm x 5 cm) at 3 x 106 cells per slide. BAEC were then grown to confluent monolayers in DMEM (Dulbecco's modified Eagle's medium) supplemented with 20% fetal bovine serum and 0.05 % fungizone and 100 U/ml streptomycin for 48 hours in 5% CO2 at 37oC (Fig. 3).

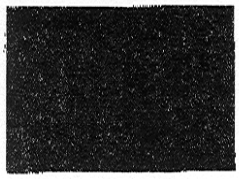


Fig. 3 EC monolayers.

Experimental Protocols Confluent monolayers of BAEC were placed in the flow channel and exposed to two flow conditions at 1 Hz for 4 hours: (1) pulsatile flow at shear stress slew rate ($\partial \tau / \partial t$ =293 dyne/(cm^{2}sec), with timeaveraged shear stress (τ ave) 50 dynes/cm2, (2) oscillating flow ($\pm$ 5 dynes/cm^2) with τ ave = 0 dynes/cm2. A positive control was performed by incubating BAECs for 4 hours with 50 μ g/ml ox-PAPC, a compound mimics MM-LDL5. Fresh monocytes were

isolated from normal volunteers with institutional review board approval and introduced to oscillatory flow. Five percent CO2 was delivered to the circulating culture medium. The pH of the cultured medium was periodically monitored with a pH probe (Fischer Scientific). The time-dependent dynamics of monocytes and BAECs interactions in response to flow conditions were captured by phase contrast microscope (Nikon), and transmitted by a CCD camera (SONY) to a TV monitor and digitalized with a video capture card (Truevision). The digital images were then analyzed by tracking algorithms to extract realtime monocytes position in relation to the EC monolayers. RNA extraction, RT-PCR and quantification of MCP-1 mRNA can be accessed on line at http://atvb.ahajournals.org[5].

3. Results

Three flow profiles representing different sites in the vascular branching points were generated: oscillatory flow and unidirectional pulsatile flow at high- and low shear stress slew rates while the ECs were being subjected to various flow patterns at 37.0 ± 0.15 oC (Fig. 4 and 5).

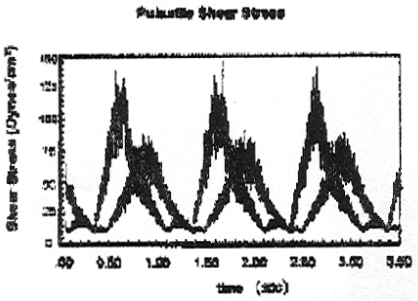


Fig. 4 Pulsatile shear stress obtained at high- vs. low shear stress slew rates. Despite the difference in slew rates, the time-averaged shear stress is 50 dynes/cm^2, and at cardiac cycle of 1 Hz for both conditions.

Four representative trajectories reflecting cellcell interactions in response to oscillating flow were shown. Figure 6 revealed the sequential pathways of an individual monocyte undergoing rolling, tumbling and, finally, firmed attachment to the endothelial cells. Figure 7 captured the instant at which two monocots were undergoing (a) attachment, (b) separation, (c) reattachment, and (d) separation while monocyte #1 eventually established anchorage on ECs at 0 reference point on the y axis. Figure 8 demonstrated monocyte-mnocyte binding while figure 9 revealed a monocyte undergoing tethering while establishing brief contact with another monocyte undergoing a zig-zag tracjectory.

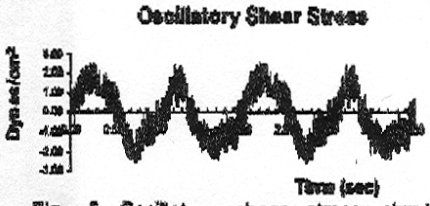


Fig. 5 Oscillatory shear stress simulating reattachment point at the arterial bifurcations.

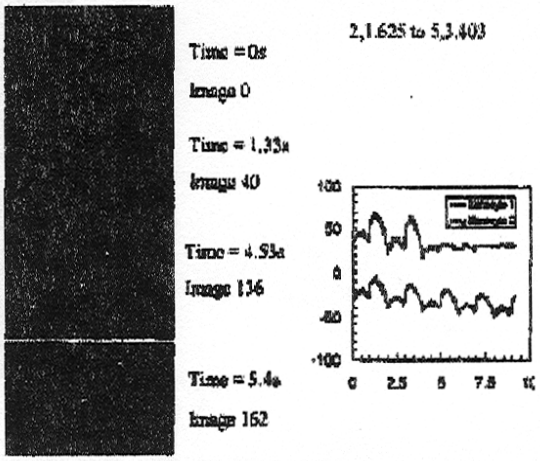
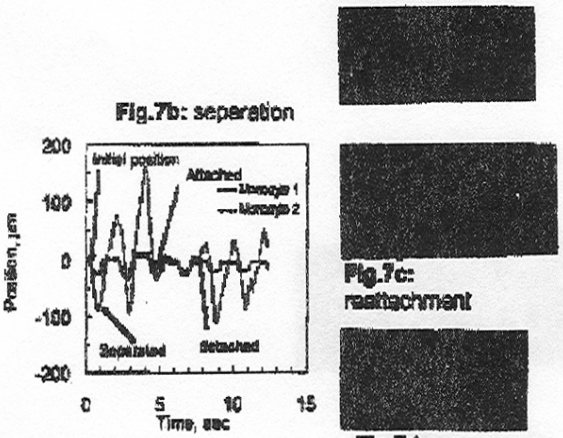


Fig. 6 Monocytes are the bright spots against the background of EC monolayers.

Fig.7(a)



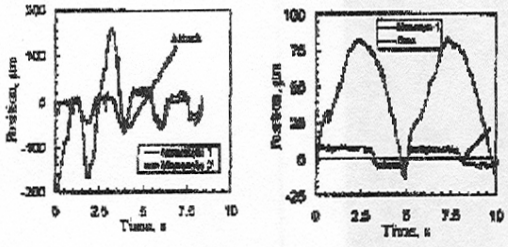


Fig. 8 Attachment Fig. 9 Tethering

MCP-1 mRNA Expression in Response to Palatial vs. Oscillatory Flow Conditions: Ox-PAPC treatment alone unregulated MCP-1 mRNA expression compared to the control (Fig.10, lane 4). Introduction of pulsatial flow at shear stress slew rates ($\partial \tau / \partial t$ = 293 dynes/cm2sec, time-averaged shear stress (τ aver) = 50 dynes/cm2) down regulated MCP-1 mRNA expression compared to no-flow conditions by 68 + 3 % (MCP-1 to GAPDH density ratio in response to shear stress slew rate $\partial \tau / \partial t$=293 dynes/cm2sec: 0.18 + 0.06; no flow condition plus ox-PAPC: 3.4 + 0.25; $P <$ 0.05, n = 5). In contrast, reversing oscillating flow unregulated MCP-1 expression by 6.6-fold compared to no flow conditions (MCP-1 to GAPDH density ratio: 4.2 + 0.25, $P <$ 0.38, n = 5) (Fig. 10). The intensity of all bands was normalized to that of GAPDH with a dose-dependent linear relation.

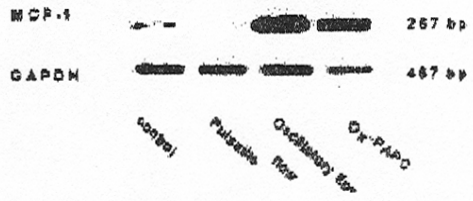


Fig. 10 EC signaling: MCP-1 mRNA expression

4. Discussion

The central theme in this paper was to understand endothelial cell sensing and signaling in response to sensing shear stress by embracing MEMS technology. Using newly designed flow system, we have captured the process of EC actuation by visualizing the history of monocyte trajectories. By understanding the forces that lead to local endothelial dysfunction, the pathophysiological mechanisms of cardiac disease might be further uncovered. The MEMS sensors offer an entry point to reveal the significant time-dependent variations in the hemodynamics of the arterial circulation under differing physiologic conditions.

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