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Spatial Mapping of Real-time Quantitative Shear Stress with Vascular Oxidative Stress

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***Abstract*—Fluid shear stress is intimately involved in vascular oxidative stress and atherosclerosis. Oxidative stress induces molecular signaling that regulates the development of vascular calcification. The explants of New Zealand White (NZW) rabbit aortas were used to assess vascular oxidative stress in non-obstructive, albeit inflammatory, lesions. The development of Micro-Electro-Mechanical Systems (MEMS) shear stress and oxidative stress sensors has provided a means to study atherogenic hemodynamics and vascular oxidative stress. Computational fluid dynamics and Doppler ultrasound were utilized in combination with the immunohistochemistry staining to show that the flow disturbance as assessed by the micro-scale sensors in non-obstructive plaques was associated with oxidative stress relevant for initiation of the arterial plaque. Our findings represent a concerted effort to assess the relationship between oxidative stress and the mechanically unstable plaque in the presence of vascular calcification.**

I. Introduction

FLUID shear stress acting on the vascular endothelial cells is intimately involved in vascular oxidative stress[1] and atherosclerosis[2]. It is recognized that low and/or oscillating shear stress, induced endothelial dysfunction and is one of the key factors in localizing early atherosclerosis, whereas laminar and/or unidirectional pulsatile flow, maintains endothelial function and is involved in compensatory remodeling. A slight plaque intrusion into the lumen results in a change in the shear stress profiles over the plaque.

Oxidative stress induces molecular signaling that regulates the development of vascular calcification[3]. It has been suggested that plaque instability is caused by a dynamic imbalance in oxidative stress, inflammatory responses and proteolytic activities[4]. Encouraging results demonstrated that hemodynamic forces applied to vascular endothelium affect the production of reactive oxygen species (ROS) as well as post-translational protein modifications[5]. Oxidized Low Density Lipoprotein (oxLDL) particles transmigrate into the subendothelial layers and induce inflammatory and proteolytic activities in the atheromas[6].

Micro-fluidic channels have been used to study the endothelial cell (EC) shape and function[7]. The development of MEMS sensors in our group has provided a means to undertake study of atherogenic hemodynamics. We hypothesize that flow disturbance as assessed by the micro-scale sensors in non-obstructive plaques is associated with oxidative stress relevant for initiation of the arterial plaque.

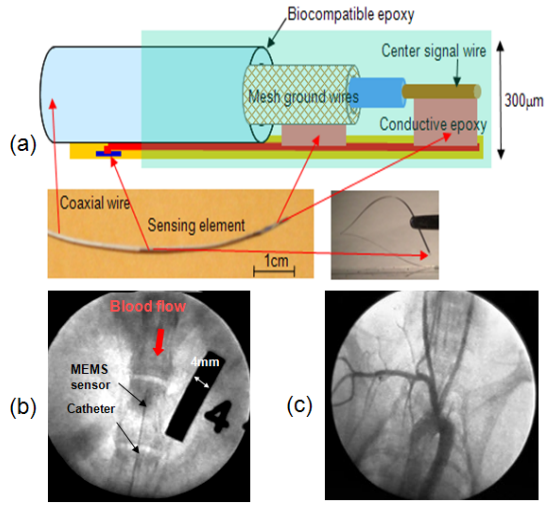

Fig. 1. (a)The flexible polymer-based sensor was bound to a coaxial wire, and the electronics were insulated without exposure to blood flow. (b)The sensor was deployed by a femoral-cut-down procedure. The position of the sensor was visualized by fluoroscope. (c)The sensor was deployed to the aortic arch, and the anatomy was delineated by contrast dye.

We developed a real-time model that addresses fluid shear stress and vascular oxidative stress using the NZW rabbits. The MEMS sensor measurement was coupled with immunohistochemistry to establish the pathophysiological

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significance of this model. Our vision is to establish early in vivo localization of arterial regions likely to develop inflamed, high-risk lesion characteristics to prevent sudden death from acute coronary syndromes or stroke.

II. Methods

A. MEMS sensor fabrication and packaging

The sensor was fabricated using surface micromachining with biocompatible materials such as parylene C, Ti and Pt. The detailed fabrication process was discussed previously[8, 9]. The sensor body was 4 cm in length, 320 μm in width and 21 μm in thickness (**Fig. 1a**). The Ti/Pt sensing element (160 μm in length by 80 μm in width) was encapsulated in parylene polymer for direct contact with the blood flow[8, 9].

MEMS sensors operate on the basis of heat transfer principle. The sensing element resides within the velocity boundary layer and the rate of heat loss from the heated sensing element to the liquid depends on the velocity profile within the boundary layer. When an electric current passes through the heated element, the change in voltage across the element is correlated with shear stress[10]. The sensors were packaged onto an electrical coaxial wire (0.4 mm in diameter) for intravascular shear stress analysis. The wire bonding sites of the Cr/Au electrode leads were connected to the coaxial wire tip using the biocompatible conductive epoxy (EPO-TEK H20E; Epoxy Technology, Billerica, MA, USA). The biocompatible epoxy (EPO-TEK 301; Epoxy Technology, Billerica, MA, USA) was applied to anchor the sensor body on the coaxial wire surface (**Fig. 1a**).

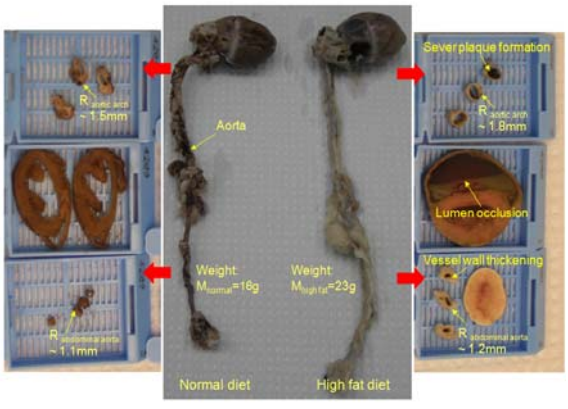

Fig. 2. Tissues isolated from adult rabbits after 8 wks on a normal diet or high fat/high cholesterol diet.

B. In Vitro Sensor Calibration and Flow Field Assessment

The wire-based sensor was introduced into a 3-D plastic tube. A range of flow rates were generated by a flow system consisting of a digital modular drive, a pump drive and a pulse dampener (Cole-Palmer Instrument Co., Vernon Hills, IL). The pump setting was calibrated using an electromagnetic flow meter (MAGFLO®, Danfoss A/S, DK). The constant temperature (CT) mode, which contains a feedback loop to keep the voltage across the sensor resistor constant, was used for real-time voltage signal acquisition. By applying various steady flow rates, the calibration curve between the voltage output cross the sensor and the shear stress was established. We used Araldite AB Epoxy Adhesive (Huntsman Advanced Materials, Basel, Switzerland) to mimic the plaque formation in the 3-D tube. An ultrasound transducer (Philips SONOS 5500) was used to observe the changes in flow fields due to the presence of the stenosis.

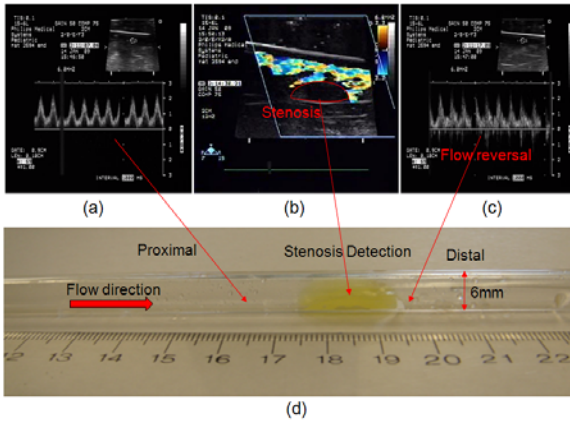

Fig. 3. Doppler ultrasound assessment of the flow field in the 3-D in vitro model with the presence of stenosis.

C. In Vivo Assessment of Intravascular Shear Stress

We have demonstrated the feasibility of acquiring real-time shear stress measurements from the NZW rabbit's aorta; specifically, abdominal aorta and aortic arch[1]. After the acute experiment as discussed in Yu et al.[8] and Ai et al.[9], a long-term follow-up study was conducted to assess dynamic changes in oxidative stress in non-obstructive plaque. The rabbits were maintained by the USC vivaria and fed on cholesterol-containing diet. After the eight-week feeding period, the same shear stress assessment procedure was performed. Using the fluoroscope in the animal angiographic lab (Phillips BV-22HQ C-arm), the operator was able to visualize and steer the coaxial wire in the arterial system.

D. Immunohisochemistry

At sacrifice, the aorta were bisected in half longitudinally and divided into segments comprising the aortic arch, thoracic aorta, and abdominal aorta (**Fig. 2**). Immuno-staining was performed for foam cells and the presence of oxLDL in the athero-prone regions in the arterial systems.

III. RESULTS

The aorta specimen showed that fat-fed rabbits had developed myocardial hypertrophy, vessel wall remodeling, and atherosclerotic plaques as evidenced by greater size and weights in abdominal aortas, left ventricles, and aortic arches **(Fig. 2)**.

Rabbit	Normal diet	High fat diet
Weight (g)	16	23
Radius of the abdominal aorta (mm)	1.1	1.2
Radius of the aortic arch (mm)	1.5	1.8

Table. 1. The changes in weight and size of the rabbit heart and artery after high fat diet.

During the *in vitro* testing, the Doppler ultrasound was used to access the flow field near the stenosis at the flow rate of 200ml/min. The changes in the velocity waveforms proximal and distal to the stenosis were recorded **(Figs. 3a and 3c)**. The pulse dampener was not included in the flow loop. Therefore, the velocity waveforms reflected the peristaltic flow generated by the pump. By using the color-Doppler flow field detection function of the ultrasound system, the tube wall and the dimensions of stenosis were delineated **(Fig. 3b)**. The colored area shown in Figure 3b indicated the lumen.

As shown in **figure 4**, the red arrows indicate the regions where oscillatory flow likely occurs; and the blue arrows indicate regions where pulsatile flow likely develops. Immuno-staining showed lesion formation in the arterial regions exposed to disturbed flow (aortic arch section). Real-time voltage signals acquired at the aortic arch showed different pattern from the voltage signal acquired at the mesentery artery level.

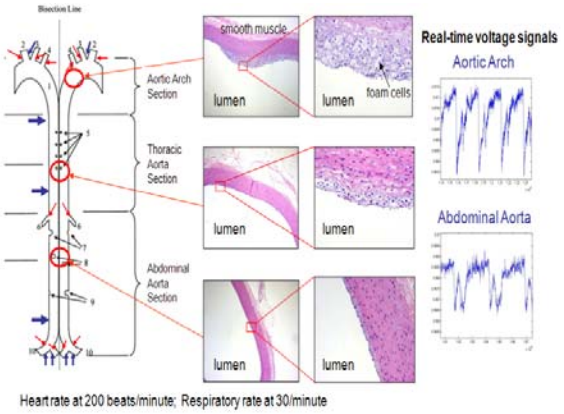

Fig. 4. The immuno-staining results and the representative voltage signals from different arterial regions.

IV. DISCUSSIONS

In this paper, we demonstrate the feasibility of using flexible polymer sensors to acquire different real-time signals from various regions in the rabbit arterial system. The effects of plaque formation were studied using both *in vitro* and *in vivo* models. This pilot study enabled us to assess the dynamic changes in intravascular shear stress in the animal models in response to hypercholesterolemic diet.

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REFERENCES

[1] Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. Arterioscler Thromb Vasc Biol. 2005;25:29-38.W.-K. Chen, *Linear Networks and Systems* (Book style). Belmont, CA: Wadsworth, 1993, pp. 123–135.

[2] Li L, Tatake RJ, Natarajan K, Taba Y, Garin G, Tai C, Leung E, Surapisitchat J, Yoshizumi M, Yan C, Abe J, Berk BC. Fluid shear stress inhibits TNF-mediated JNK activation via MEK5-BMK1 in endothelial cells. Biochem Biophys Res Commun. 2008;370:159-163.

[3] Byon CH, Javed A, Dai Q, Kappes JC, Clemens TL, Darley-Usmar VM, McDonald JM, Chen Y. Oxidative stress induces vascular calcification through modulation of the osteogenic transcription factor Runx2 by AKT signaling. J Biol Chem. 2008;283:15319-15327.

[4] Griendling KK FG. Oxidative stress and cardiovascular injury: Part II: animal and human studies. Circulation. 2003;108:2034-2040.

[5] Hsiai T, Berliner JA. Oxidative stress as a regulator of murine atherosclerosis. Curr Drug Targets. 2007;8:1222-1229.

[6] Witztum JR. The oxidation hypothesis of atherosclerosis. Lancet. 1994;344:793-795.

[7] Gray BL, Lieu DK, Collins SD, Smith RL, Barakat AI. Microchannel platform for the study of endothelial cell shape and function. Biomedical Microdevices. 2002;4:9-16.

[8] Yu H, Ai, L., Rouhanizadeh, M., Patel, D., Kim, E.S., Hsiai T.K. . Flexible Polymer Sensors for In Vivo Intravascular Shear Stress Analysis IEEE/ASME J. MEMS. 2008;17:1178-1186.

[9] Ai L, Yu H, Dai W, Hale SL, Kloner RA, Hsiai TK. Real-time intravascular shear stress in the rabbit abdominal aorta. IEEE Trans Biomed Eng. 2009;56:1755-1764.

[10] Lin Q, Jiang FK, Wang XQ, Xu Y, Han ZG, Tai YC, Lew J, CM. H. Experiments and simulations of MEMS thermal sensors for wall shear-stress measurements in aerodynamic control applications. Journal of Micromechanics and Microengineering. 2004;14:1640-1649.