Published in final edited form as:

Biosens Bioelectron. 2013 May 15; 43: 237–244. doi:10.1016/j.bios.2012.12.024.

Elevated Electrochemical Impedance in the Endoluminal Regions with High Shear Stress: Implication for Assessing Lipid-Rich Atherosclerotic Lesions

Fei Yu¹, Juhyun Lee¹, Nelson Jen¹, Xiang Li¹, Qian Zhang², Rui Tang³, Qifa Zhou¹, Eun. S. Kim², and Tzung K. Hsiai¹

¹Department of Biomedical Engineering and Cardiovascular Medicine, University of Southern California, Los Angeles, CA

²Department of Electrical Engineering, University of Southern California, Los Angeles, CA

³Department of Electrical Engineering, Arizona State University, Phoenix, AZ

Abstract

Background—Identifying metabolically active atherosclerotic lesions remains an unmet clinical challenge during coronary intervention. Electrochemical impedance (EIS) increased in response to oxidized low density lipoprotein (oxLDL)-laden lesions. We hereby assessed whether integrating EIS with intravascular ultrasound (IVUS) and shear stress (ISS) provided a new strategy to assess oxLDL-laden lesions in the fat-fed New Zealand White (NZW) rabbits.

Methods and Results—A micro-heat transfer sensor was deployed to acquire the ISS profiles at baseline and post high-fat diet (HD) in the NZW rabbits (n=8). After 9 weeks of HD, serum oxLDL levels (mg/dL) increased by 140-fold, accompanied by a 1.5-fold increase in kinematic viscosity (cP) in the HD group. Time-averaged ISS (ISS_{ave}) in the thoracic aorta also increased in the HD group (baseline: 17.61±0.24 vs. 9 weeks: 25.22±0.95 dyne/cm², n=4), but remained unchanged in the normal diet group (baseline: 22.85±0.53 dyne/cm² vs. 9 weeks: 22.37±0.57 dyne/cm², n=4). High-frequency Intravascular Ultrasound (IVUS) revealed atherosclerotic lesions in the regions with augmented ISS_{ave}, and concentric bipolar microelectrodes demonstrated elevated EIS signals, which were correlated with prominent anti-oxLDL immuno-staining (oxLDL-free regions: 497±55 Ω, n = 8 vs. oxLDL-rich lesions: 679±125 Ω, n = 12, *P* < 0.05). The equivalent circuit model for tissue resistance between the lesion-free and ox-LDL-rich lesions further validated the experimental EIS signals.

Conclusions—By applying electrochemical impedance in conjunction with shear stress and high-frequency ultrasound sensors, we provided a new strategy to identify oxLDL-laden lesions. The study demonstrated the feasibility of integrating EIS, ISS, and IVUS for a catheter-based approach to assess mechanically unstable plaque.

© 2012 Elsevier B.V. All rights reserved

Corresponding Author: Tzung K. Hsiai, M.D., Ph.D. Department of Biomedical Engineering and Division of Cardiovascular Medicine University of Southern California Los Angeles, CA 90089 hsiai@usc.edu Telephone: (213)740-7236 Fax: (213) 740-0343.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosures None

Keywords

Shear Stress; Electrochemical Impedance; Intravascular ultrasound; OxLDL; Atherosclerotic lesions

1. Introduction

Metabolically active lesions is of clinical significant in the development of mechanically unstable plaque.(Naghavi and Falk 2010) Tissue impedance spectroscopy is an emerging electrochemical strategy to characterize atherosclerotic lesions.(Streitner et al. 2012; Streitner et al. 2009; Yu et al. 2011b) Elevated electrochemical impedance spectroscopy (EIS) signals are associated with metabolically active lesions in both *ex vivo* (Hsiai et al. 2002; Li et al. 2012a) and *in vivo* models.(Konings et al. 1997; Streitner et al. 2012; Streitner et al. 2009; Suselbeck et al. 2005; Yu et al. 2011b; Yu et al. 2011c)_ENREF_24 When the microelectrodes are in contact with endoluminal surface, EIS signals are reproducible and independent of lumen diameters, blood viscosity, and the flow rate.(Yu et al. 2011b) For this reason, we integrated EIS with high-frequency ultrasound and shear stress to assess lipid-laden atherosclerotic lesions

Currently, gray-scale intravascular ultrasound (IVUS) is deemed as the gold standard for *in vivo* imaging of the vessel walls.(García-García et al. 2011) While IVUS has enabled quantitative assessment of coronary artery and peripheral vascular disease, assessing thin-capped fibrous atheromas has been hampered by its gray-scale representation of the artery wall and its limited spatial resolution.(García-García et al. 2011) By integrating EIS with high-frequency IVUS at a sampling rate of 400 MHz, we proposed to distinguish thick-versus thin-capped fibrous atheromas that harbor active lipids; namely, oxidized low density lipoprotein (oxLDL).

Fluid shear stress imparts mechano-signal transduction that is intimately linked with the initiation and development of atherosclerosis.(Ai et al. 2010; Bark Jr and Ku 2010; Chatzizisis et al. 2007; Cheng et al. 2007; Hsiai et al. 2002; Stone et al. 2003) Both the spatial (τ/x) and temporal (τ/t) components of shear stress modulate the focal nature of vascular oxidative stress in the promotion of pro-inflammatory states and post-translational LDL oxidative modification. (Hsiai et al. 2002; Nerem et al. 1998; Sun et al. 2007) In the athero-prone regions, LDL particles transmigrate into the subendothelial layers, (Sun et al. 2007) where post-translational oxidative modifications of LDL particles induce activation of matrix metalloproteinases (Li et al. 2012b; Madamanchi et al. 2005) and up-regulation of NF- κ B-mediated adhesion molecules to destabilize the plaque. (Hwang et al. 2003; Lee et al. 2012; Li et al. 2012a; Madamanchi et al. 2005; Parathath et al. 2011)

In this context, we sought to demonstrate oxLDL-laden lesions by integrating hemodynamic, imaging, and electrochemical approach. We established increased time-averaged intravascular shear stress (ISS_{ave}) in response to high fat diets in the NZW rabbit model. After 9 weeks, IVUS enabled visualization of endoluminal regions that harbored atherosclerotic lesions for the assessment of EIS signals by the concentric bipolar microelectrodes. Histology analysis for prominent anti-oxLDL lesions provided a validation for the elevated EIS signals. Hence, we introduced an integrated approach to enhance the characterization of oxLDL-laden atherosclerotic lesions with a translational implication for mechanically unstable plaque.

2. Materials and Methods

Microfabrication and Calibration of Catheter-Based Flexible Polymer Sensors

The intravascular thermal sensor was fabricated using surface micromachining techniques as previously described (Yu et al. 2008). The Ti/Pt sensing element (240 μm in length and 80 μm in width) was encapsulated in parylene C polymer for direct contact with the blood flow. All materials used, including Parylene C, Ti, and Pt, offered a high level of biocompatibility for *in vivo* investigations.

The concentric bipolar microelectrodes for electrochemical impedance spectroscopy (EIS) measurements sensor were fabricated using a similar surface micromachining technique; chromium deposition was used instead of the Ti/Pt for the sensing electrodes. The concentric bipolar microelectrodes consisted of a working and a counter electrode; the former was the inner pole with a diameter of 100 μm , and the latter was the outer ring-shaped electrode with an outer diameter of 500 μm , and a width of 100 μm . The spacing between the inner and outer electrodes was 100 μm (Figures 1a and 1b).

Both the intravascular thermal sensors and concentric bipolar microelectrodes were integrated to an electrical coaxial wire of 0.4 mm in diameter (Tyco Electronics, Berwyn, PA) as a guide wire for intravascular deployment and interrogation. The bonding sites of the sensors were connected to the terminal end of the coaxial wire leads using conductive epoxy (EPO-TEK H20E; Epoxy Technology, Billerica, MA). Biocompatible epoxy (EPO-TEK 301; Epoxy Technology, Billerica, MA) anchored the sensor body onto the coaxial wire surface (Supplemental Figure 1).

In vivo Assessment of Intravascular Thermal Profiles in the New Zealand White Rabbit Model

We acquired intravascular thermal profiles in the descending, thoracic, and peri-renal abdominal aortas of NZW rabbits, and the data were calibrated to ISS as an approximation of wall shear stress (Ai et al. 2009; Yu et al. 2011a) (Supplemental Figure 2). All *in vivo* animal experiments were performed at the Heart Institute of the Good Samaritan Hospital (Los Angeles, CA) with approval from its Institutional Animal Care and Use Committee (IACUC). Deployment of the flexible MEMS sensors into the rabbit's aorta was performed in compliance with the IACUC approved protocol. Eight age-matched male NZW rabbits (ten weeks, mean body weight 2442 ± 210 g) were acquired from a local breeder (Irish Farms, Norco, CA) and maintained in the Good Samaritan Hospital Vivarium. After a seven-day quarantine period, the rabbits were anesthetized through an intramuscular injection of 50 mg/kg ketamine (JHP Pharmaceuticals, LLC) combined with 10 mg/kg xylazine (IVX Animal Health, Inc.). The animals were anticoagulated with heparin (100 units/kg) prior to the sensor deployment.

To obtain artery dimensions and blood flow rates in the rabbit aortas, we positioned the ultrasound transducer (Philips SONOS 5500) over the abdomen to interrogate the arterial blood flow prior to sensor deployment. Continuous blood pressure measurements were recorded with an automated tail cuff (IITC/Life Science Instruments). In the animal angiographic laboratory, fluoroscope (Phillips BV-22HQ C-arm) and contrast dye injection enabled us to localize the position of the sensors in relation to the inner aortic diameter, allowing for steering the catheter-based sensors.

Catheter-based thermal sensor was deployed into rabbit aorta via left femoral artery cut-down and advanced into abdominal, thoracic and descending aorta for respective thermal profile assessment with the aid of fluoroscopy to guide the positioning of the catheter (Yu et al. 2011a). Constant temperature (CT) circuit was used to drive the thermal sensors for real-

time voltage signal acquisition (Rouhanizadeh et al. 2005). The voltage across the sensing element was monitored at a sampling rate of 2000 Hz by a LabVIEW-based data acquisition system, including a data acquisition board (USB-6216 DAQ device, National Instruments, Austin, TX) and a laptop computer (ThinkPad T61, Lenovo, China). Signal processing, wavelet decomposition and low-pass filters were applied to minimize the background noise (Sun et al. 2009). After the baseline intravascular thermal profile assessment, the femoral artery was resutured and skin was closed with staples to allow recovery. Buprenorphine, an analgesic was administered at 0.02 mg/kg during the first week as needed after baseline measurements. The rabbits were then randomly divided into 2 groups: 1) normal standard chow diet (ND) (n = 4); and 2) hypercholesterolemic diet (HD) (n = 4) containing 1.5% cholesterol & 6% peanut oil (Newco®, CA). After 9 weeks, ISS measurements were repeated with the identical experiment protocols. The rabbits were then sacrificed and their aortas were isolated for *ex vivo* assessments and histological evaluations.

Computational Fluid Dynamics (CFD) Simulation

CFD code was developed to compare between analytical and experimental data. Three-dimensional modeling of rabbit aortic geometries (aortic arch, thoracic, abdominal, renal aorta) was reconstructed by Solidworks (Concord, Massachusetts, USA). The size of individual aortic segments was obtained from angiographic and ultrasound imaging. The inlet velocity profiles were acquired by the pulsed-wave Doppler velocity measurements. The outlet boundary condition was determined from the mean arterial pressure obtained during ISS experiments. Geometries were meshed from Solidworks flow simulation. After defining the boundary conditions and performing geometry meshing, meshed models were solved. The governing equations were solved by assuming laminar, incompressible, and unsteady flow under the non-slip condition.

High-Frequency Intravascular Ultrasound Imaging (IVUS) of NZW Rabbit Aortas

IVUS imaging of the rabbit aorta explants was performed using a custom-built ultrasound imaging system within 4 hours after rabbits were sacrificed and the aortas were isolated (Wei et al. 2011). Segments approximately 2 cm in length were cut sequentially along the aorta and maintained in Dulbecco's Modified Eagle Medium (DMEM) for cell viability. Individual segments were then vertically positioned in a container placed on top of a rotating platform. The ultrasound transducer was introduced into the aortic lumen from top along the central axis of the rotating platform to achieve rotational scanning. Two-way pulse-echo measurement was performed using a single pulser/receiver unit (Olympus NDT, Inc., Kennewick, WA). The detected ultrasound echo signals were digitized by a 12 bit data acquisition board (Gage Applied Technologies, Lockport, IL) operating at the sampling rate of 400 MHz. A function generator provided a 2.5 KHz trigger signal to the pulser/receiver and the data acquisition board. Gross histology of atherosclerotic lesions was identified both visually and from the IVUS images, and the positions of the lesions with respect to the aorta segment geometry were labeled for the corresponding electrochemical impedance and immunohistochemistry.

Electrochemical Impedance Spectroscopy Assessment of NZW Rabbit Aortas

Immediately after IVUS assessment, the rabbit aorta segments were flushed with and maintained in phosphate buffered saline (PBS) for EIS assessment. Endoluminal EIS measurements were performed by using the concentric bipolar microelectrodes at multiple sites associated with the atherosclerotic lesions and compared with the adjacent healthy endoluminal regions (Yu et al. 2011b). To ensure contact between the EIS sensor and the endoluminal measurement sites on the arterial wall, we mounted the microelectrodes to a steerable guide wire and made contact with the atherosclerotic lesions by steering the

terminal end of guide wire. An Ag/AgCl electrode immersed in the PBS solution was used as a reference electrode. EIS measurements were performed by using a Gamry Series G 300 potentiostat (Gamry Instruments, PA) installed in a desktop computer. An input of 10 mV peak-to-peak AC voltage with a frequency decay ranging from 300 kHz to 100 Hz was delivered to the sites. The magnitudes and phases of the impedance were acquired at 20 data points per frequency decade. After the measurements, the electrical resistance properties of the tissue were calculated on the basis of equivalent circuit model by using the Gamry Echem Analyst software suite (Gamry Instruments, PA) as previously described. (Yu et al. 2011b)

Immunohistochemistry

Vascular rings corresponding to the EIS measurement sites were cut from the aorta segments, and immersed in 4% paraformaldehyde. After 24 hours, they were embedded in paraffin and cut into serial 5- μ m sections. Immunostaining was performed with standard techniques in paraffin embedded vascular tissue using biotinylated secondary antibodies and streptavidin-conjugated horse radish peroxidase (HRP). OxLDL was stained with mAb4E6 antibody. (Holvoet et al. 1998) Tissue sections were imaged (Olympus IX70 microscope, Japan), and were captured with a CCD digital camera (ProgRes C3, Jenoptik, Germany).

Statistical Analysis

Data were expressed as means $\pm$ SD where it was applicable. A student's *t*-test was performed for statistical comparisons between two groups of values. One-way analysis of variance (ANOVA) was performed for comparisons of multiple groups of values. The Tukey procedure was performed to determine the statistical significance among multiple groups. A *P* value of < 0.05 was considered statistically significant.

3. Results

OxLDL-Laden Lesions in the Endoluminal Regions Exposed to Augmented ISS

Representative ISS profiles were validated with the CFD simulations for wall shear stress (WSS) (Fig. 2), the time-averaged ISS (ISS_{ave}) and peak ISS were comparable with the computed WSS values consistent with the established 23% and 14% experimental errors (Ai et al. 2009; Ai et al. 2010), respectively. The acquired ISS profiles were then compared between baseline and after 9 weeks of high-fat diet (Fig. 3, 4) (Supplemental Figure 4 and 5 for the entire aorta).

In the normal diet-fed arm, ISS_{ave} values increased from distal aortic arch to infra-renal aorta in relation to the tapering in the diameters of downstream vessels. However, there was no significant changes in ISS profiles and the ISS_{ave} values between the baseline and after 9 weeks ($n=4$, $P > 0.05$) (Fig. 3c). The corresponding cross-section of the thoracic aorta visualized by the high-frequency IVUS revealed no gross endoluminal lesions in agreement with the negative immunostaining for anti-oxLDL throughout the entire aorta (Fig. 3d).

In the fat-fed arm, the magnitude of ISS profiles increased after 9 weeks compared to the baseline in the representative peri-renal aorta (Fig. 4). Similar to the trend in normal diet-fed rabbits, ISS_{ave} values increased from the distal aortic arch to the infra-renal aortas (Fig. 4c). However, there was a significant augmentation in ISS throughout the entire regions after 9 weeks ($n=4$, $P < 0.05$), accompanied by a greater standard deviations among individual measurements than those of the baseline. ISS_{ave} values increased by 24%, 20%, 30%, and 38% in the distal aortic arch, thoracic, abdominal, and infra-renal aorta, respectively ($n=4$, $P < 0.05$) (Fig. 4c and Supplement Table 1). Similarly, the peak ISS values increased by 28%, 16%, 32%, and 26%, respectively ($n=4$, $P < 0.05$) (Supplement Table 1). IVUS imaging of

the thoracic aorta section revealed endoluminal lesions that were prominent for anti-oxLDL staining (Fig. 4d). In parallel, serum LDL increased by 140-fold ($n=4$, $P < 0.05$), accompanied by an increase in kinematic viscosity by 1.5-fold ($n=4$, $P < 0.05$) after 9 weeks (Fig. 5). Hence, augmented ISS_{ave} values in response to high-fat diets were associated with endoluminal evidence of atherosclerotic lesions as supported by IVUS and immunohistochemistry.

Increased Electrochemical Impedance in Association with OxLDL-Laden Lesions

Under the IVUS guidance, concentric bipolar microelectrodes were deployed to assess EIS signals. The frequency-dependent EIS spectra were significantly elevated from 30 kHz to 100 kHz in the oxLDL-laden regions, as evidenced by immuno-staining (Fig. 6a, 6e and 6f) (Supplementary Figure 6). Significant differences in phase spectra also developed from 10 kHz to ~30 kHz (Fig. 6b). The impedance spectra were subsequently input into the equivalent circuit model to simulate tissue resistance between the lesion-free and ox-LDL-rich lesions as previously reported (Fig. 6c). (Yu et al. 2011b) Both simulated impedance spectra and experimental data were in close agreement associated with a minimal deviation (1% to 5%).

In the equivalent circuit model, the constant phase element (CPE) is associated non-ideal double layer capacitance at electrode/tissue interface and is defined to have impedance of

$$Z_{CPE} = \frac{1}{Y\omega^a} \quad (1)$$

where Y is the empirical admittance and a is an empirical constant phase value between 0 and 1 (Hleli et al. 2006). Our fitted CPE constants Y and a for healthy rabbit aorta ($n = 8$) and plaques ($n = 12$) are 104 ± 49 nS, 83 ± 31 nS, and 0.727 ± 0.089 , 0.781 ± 0.095 , respectively. We observed no significant difference in Y and a values between healthy rabbit aorta wall and lesion sites ($p > 0.05$). The calculated tissue resistance for the oxLDL-rich lesion ($679 \pm 125 \Omega$, $n = 12$) was significantly higher in comparison with that of lesion-free regions ($497 \pm 55 \Omega$, $n = 8$) (Fig. 6f).

4. Discussion

The key findings in the current study are to demonstrate an integrated strategy with a combination of three micro-sensors to assess endoluminal tissue impedance in the regions of augmented shear stress as visualized by the high-frequency ultrasound imaging. Identification of the distinct frequency-dependent EIS signals in the oxLDL-rich lesions were made possible by the newly developed flexible concentric bipolar microelectrodes (Figure 1a). In our current study, despite of having a limitation of small sample size, we demonstrated augmented ISS values in response to high-fat diet by deploying the flexible micro-thermal sensors to the aorta of NZW rabbits. Interrogation of the regions harboring augmented shear stress by IVUS revealed atherosclerotic lesions. Elevated EIS signals in these lesions were associated with prominent anti-oxLDL staining. Hence, our approach provides an experimental basis to further integrate the three sensors for simultaneous characterization of mechanically unstable plaque.

Intravascular electrochemical impedance spectroscopy is an emerging technology capable of differentiating cellular composition in the atherosclerotic plaques, thus offering a feasible strategy to identify metabolically active and mechanically unstable lesions. (Streitner et al. 2012; Streitner et al. 2009; Yu et al. 2011b; Yu et al. 2011c) We and others have established a quantitative correlation between tissue impedance and atherosclerotic lesions in terms of active lipid content (oxLDL). Streiners *et al.* deployed impedance sensor by using a linear 4-

point electrode configuration into *ex vivo* human coronary arteries, and demonstrated that inflammatory process in advanced vulnerable plaques (Type V) engendered an elevated tissue impedance.(Streitner et al. 2012) However, the linear 4-electrode configuration poses two main constraints: 1) the large size of 4-electrode array (1.4mm in the entire length) limits its capability to assess large lesions; and 2) the linear-arrays entails a limited spatial resolution to assess small and non-homogeneous lesions. For these reasons, we proposed the concentric bipolar microelectrodes to provide high spatial resolution (0.5mm diameter) and symmetric tissue impedance. Using our concentric bipolar microelectrodes, we distinguished pre-atherosclerotic lesions that harbored oxLDL and foam cell infiltrates in the descending aorta immediately distal the aortic arch.(Yu et al. 2011b) Unlike the linear 4-point configuration, we proposed the use of concentric bipolar microelectrodes to address uneven lesion topography, heterogeneous tissue composition and non-uniform current distribution; thus, allowing for impedance assessment independent of sensor orientation at a high spatial resolution. Furthermore, the compact size of the concentric configuration allows for packaging of an array of concentric bipolar electrodes onto a balloon catheter for mapping the endoluminal impedance; thus, enhancing specificity and sensitivity of identifying active lipid-rich lesions.

In parallel, we developed equivalent circuit model and performed simulation to isolate the impedance contribution by the lesion tissue R_B (Fig. 6c). We utilized a modified Randle's circuit to account for the non-Faraday impedance at tissue-electrode interface and tissue non-homogeneities.(Franks et al. 2005; Hleli et al. 2006). In our model, the CPE component is primarily associated with electrode surface properties and surface/solute interactions. We observed no significant difference in CPE values between healthy rabbit aorta wall and lesion sites measured with the same concentric bipolar electrodes setup, indicating that the electrode/tissue interface impedance did not change significantly due to lesion development. The value of tissue resistance component R_B is largely dependent on its water content, electrolyte concentration, as well as the presence of oxidative/inflammatory substances and calcification. Applying computational simulation, we were able to demonstrate a significantly higher tissue resistance in the oxLDL-rich lesions. Hence, elevated EIS signals in the regions of augmented ISS_{ave} helped identify active lipid-rich lesions in which oxidized LDL and macrophage-derived foam cells are the important pro-inflammatory components.(Schwartz et al. 2007)

By the integrated micro-sensor approach, we further demonstrated augmented intravascular shear stress (ISS) in response to high-fat diet. Multiple factors were implicated in the ISS augmentation; namely, changes in the compliance of vasculature, endoluminal remodeling, increased blood viscosity, and presence of atherosclerotic lesions. Hypercholesterolemia is well-recognized to reduce arterial compliance.(Giannattasio et al. 2001) Reduction in coronary artery compliance in patients with early atherosclerosis is associated with endothelial dysfunction and an increase in wall shear stress.(Takumi et al. 2010) While compensatory remodeling of coronary vasculature initially maintains shear stress, (Dolan et al. 2012; Huo and Kassab 2012; Samady et al. 2011) remodeling-mediated neointimal hyperplasia is conducive to the development of high shear stress in response to reduced vessel lumen diameters.(Zilla et al. 2012) The presence of pre-atherosclerotic lesions further engenders disturbed flow downstream to the stenosis, (Varghese and Frankel 2003; Zhang 2009) recruiting monocyte and LDL particles transmigration into the subendothelial layers (Ai et al. 2009; Davies et al. 1986) as well as promoting atherothrombosis.(Bark Jr and Ku 2010) Using the ApoE knockout mouse model with the constrictive carotid artery collars, Ding et al. reported elevated shear stress within the constrictive regions and low shear stress at proximal regions coupled with endothelial disfunction(Ding et al. 2010). In corollary, we demonstrated augmented intravascular shear stress after 9 weeks of high-fat diet due to both presence of endoluminal lesions and a significant increase in blood viscosity. Using the

MEMS thermal sensors, we have also reported that low shear stress developed at the proximal region or upstream from the stenotic lesions. (Ai et al. 2010) Our current protocol did not result in high-degree stenotic lesions. However, we also observed that low time-averaged shear stress developed in the arterial branching points or curvatures where atherosclerosis preferentially occurred. Finally, augmented in ISS in fat-fed rabbits was also influenced by blood viscosity. In the current study, high-fat diet resulted in a 30.5% higher in blood viscosity than that of normal diet (4.23cP vs. 2.94cP), accompanied by a 138.67-fold increase in serum LDL concentration after 9 weeks (26.2 mg/dL to 3659.4 mg/dL). In contrast, LDL concentrations in the normal diet decreased by 67% after 9 weeks (from 15.8 mg/dL to 5.2 mg/dL).

In conjunction with intravascular ultrasound (IVUS), electrochemical impedance approach further holds promises to distinguish between thick- and thin-capped fibrous plaques associated with a large lipid pool. Although other imaging modalities, including X-ray angiography, are able to identify the plaque morphology, characterization of lipid-rich and mechanically vulnerable plaque remains a clinical challenge. Thus, we demonstrated that integrating IVUS and EIS signals afforded real-time and *in vivo* strategy to identify mechanically unstable plaque, and additional investigations with larger sample size are needed to provide a robust statistical power for the pre-clinical studies.

5. Conclusion

In this study, elevated tissue impedance in the endoluminal regions of augmented shear stress were demonstrated in the fat-fed NZW rabbit model. Our integrated approach revealed two new findings: 1) time-averaged ISS increased in the regions of atherosclerotic lesions as visualized by high-frequency IVUS, and 2) the elevated EIS signals in these lesions were associated with active lipid content. In this context, integrating intravascular ultrasound imaging, hemodynamics and tissue impedance offer a translational basis for combining the three micro-sensors for diagnostic applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to express gratitude to Wangde Dai., Sharon L. Hale and Dr. Robert A. Kloner at the Heart Institute of Good Samaritan Hospital, Los Angeles for the rabbit protocol. The authors are grateful for the technical assistance by Dr. Hongyu Yu and Yongmo Yang at Department of Electrical Engineering, Arizona State University.

Funding Sources This project was supported by National Heart, Lung, and Blood Institute (NHLBI) 83015 (TKH) and NHLBI 091302 (TKH), and American Heart Association 11PRE7370088 (FY).

References

- Ai L, Yu H, Takabe W, Paraboschi A, Yu F, Kim E, Li R, Hsiai TK. Journal of biomechanics. 2009; 42(10):1429–1437. [PubMed: 19457490]
- Ai L, Zhang L, Dai W, Hu C, Kirk Shung K, Hsiai TK. Journal of biomechanics. 2010; 43(14):2678–2683. [PubMed: 20655537]
- Bark DL Jr, Ku DN. Journal of biomechanics. 2010; 43(15):2970–2977. [PubMed: 20728892]
- Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Journal of the American College of Cardiology. 2007; 49(25):2379–2393. [PubMed: 17599600]

- Cheng C, Tempel D, van Haperen R, de Boer HC, Segers D, Huisman M, van Zonneveld AJ, Leenen PJ, van der Steen A, Serruys PW, de Crom R, Krams R. *Journal of Clinical Investigation*. 2007; 117(3):616–626. [PubMed: 17304353]
- Davies PF, Remuzzi A, Gordon EJ, Dewey CF, Gimbrone MA. *Proceedings of the National Academy of Sciences*. 1986; 83(7):2114.
- Ding SF, Ni M, Liu XL, Qi LH, Zhang M, Liu CX, Wang Y, Lv HX, Zhang Y. *American Journal of Physiology-Heart and Circulatory Physiology*. 2010; 298(6):H2121–H2129. [PubMed: 20382850]
- Dolan JM, Sim FJ, Meng H, Kolega J. *American Journal of Physiology-Cell Physiology*. 2012; 302(8):C1109–C1118. [PubMed: 22173868]
- Franks W, Schenker I, Schmutz P, Hierlemann A. *IEEE Transac Biomed Eng*. 2005; 52(7):1295–1302.
- García-García HM, Gogas BD, Serruys PW, Bruining N. *The International Journal of Cardiovascular Imaging (formerly Cardiac Imaging)*. 2011; 27(2):215–224.
- Giannattasio C, Failla M, Emanuelli G, Grappiolo A, Boffi L, Corsi D, Mancina G. *Hypertension*. 2001; 38(5):1177–1180. [PubMed: 11711518]
- Hleli S, Martelet C, Abdelghani A, Bessueille F, Errachid A, Samitier J, Hays H, Millner P, Burais N, Jaffrezic-Renault N. *Materials Science and Engineering: C*. 2006; 26(2):322–327.
- Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. *Circulation*. 1998; 98(15):1487–1494. [PubMed: 9769301]
- Hsiai TK, Cho SK, Honda HM, Hama S, Navab M, Demer LL, Ho CM. *Annals of biomedical engineering*. 2002; 30(5):646–656. [PubMed: 12108839]
- Huo Y, Kassab GS. *Journal of Hypertension*. 2012; 30(3):608. [PubMed: 22252479]
- Hwang J, Michael H, Salazar A, Lassegue B, Griendling K, Navab M, Sevanian A, Hsiai TK. *Circulation research*. 2003; 93(12):1225–1232. [PubMed: 14593003]
- Konings MK, Mali W, Viergever MA. *IEEE transactions on medical imaging*. 1997; 16(4):439–446. [PubMed: 9263001]
- Lee S, Springstead JR, Parks B, Romanoski CE, Palvolgyi R, Ho T, Nguyen P, Lusis AJ, Berliner JA. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2012; 32(5):1246–1254.
- Li JJ, Meng X, Si HP, Zhang C, Lv HX, Zhao YX, Yang JM, Dong M, Zhang K, Liu SX. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2012a; 32(5):1158–1166.
- Li R, Mittelstein D, Lee J, Fang K, Majumdar R, Tintut Y, Demer LL, Hsiai TK. *American Journal of Physiology-Cell Physiology*. 2012b; 302(4):C658–C665. [PubMed: 22031601]
- Madamanchi NR, Vendrov A, Runge MS. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2005; 25(1):29–38.
- Naghavi M, Falk E. *Asymptomatic Atherosclerosis*. 2010:13–38.
- Nerem RM, Alexander RW, Chappell DC, Medford RM, Varner SE, TAYLOR WR. *The American journal of the medical sciences*. 1998; 316(3):169. [PubMed: 9749558]
- Parathath S, Mick SL, Feig JE, Joaquin V, Grauer L, Habiell DM, Gassmann M, Gardner LB, Fisher EA. *Circulation research*. 2011; 109(10):1141–1152. [PubMed: 21921268]
- Rouhanizadeh M, Lin TC, Arcas D, Hwang J, Hsiai TH. *Annals of Biomedical Engineering*. 2005; 33(10):1360–1374. [PubMed: 16240085]
- Samady H, Eshtehardi P, McDaniel MC, Suo J, Dhawan SS, Maynard C, Timmins LH, Quyyumi AA, Giddens DP. *Circulation*. 2011; 124(7):779–788. [PubMed: 21788584]
- Schwartz SM, Galis ZS, Rosenfeld ME, Falk E. *Arterioscler Thromb Vasc Biol*. 2007; 27(4):705–713. [PubMed: 17332493]
- Stone PH, Coskun AU, Kinlay S, Clark ME, Sonka M, Wahle A, Ilegbusi OJ, Yeghiazarians Y, Popma JJ, Orav J. *Circulation*. 2003; 108(4):438–444. [PubMed: 12860915]
- Streitner I, Goldhofer M, Cho S, Kinscherf R, Thielecke H, Borggreffe M, Süselbeck T, Streitner F. *PLoS ONE*. 2012; 7(4):e35405. [PubMed: 22509411]
- Streitner I, Goldhofer M, Cho S, Thielecke H, Kinscherf R, Streitner F, Metz J, Haase KK, Borggreffe M, Süselbeck T. *Atherosclerosis*. 2009; 206(2):464–468. [PubMed: 19419719]
- Sun N, Wood NB, Hughes AD, Thom SAM, Xu XY. *Annals of biomedical engineering*. 2007; 35(10):1782–1790. [PubMed: 17629792]

- Sun P, Zhang Y, Yu F, Parks E, Lyman A, Wu Q, Ai L, Hu CH, Zhou Q, Shung K, Lien CL, Hsiai TK. *Ann Biomed Eng.* 2009; 37(5):890–901. [PubMed: 19280341]
- Suselbeck T, Thielecke H, Kochlin J, Cho S, Weinschenk I, Metz J, Borggreffe M, Haase KK. *Basic research in cardiology.* 2005; 100(5):446–452. [PubMed: 15795794]
- Takumi T, Yang EH, Mathew V, Rihal CS, Gulati R, Lerman LO, Lerman A. *Heart.* 2010; 96(10): 773–778. [PubMed: 20448128]
- Varghese SS, Frankel SH. *Journal of biomechanical engineering.* 2003; 125:445. [PubMed: 12968569]
- Wei W, Li X, Zhou Q, Shung KK, Chen Z. *Journal of biomedical optics.* 2011; 16:106001. [PubMed: 22029348]
- Yu F, Ai L, Dai W, Rozengurt N, Yu H, Hsiai TK. *Annals of biomedical engineering.* 2011a; 39(6): 1736–1744. [PubMed: 21380571]
- Yu F, Dai X, Beebe T, Hsiai T. *Biosensors and Bioelectronics.* 2011b; 30(1):165–173. [PubMed: 21959227]
- Yu F, Li R, Ai L, Edington C, Yu H, Barr M, Kim E, Hsiai TK. *Annals of biomedical engineering.* 2011c; 39(1):287–296. [PubMed: 20652746]
- Yu H, Ai L, Rouhanizadeh M, Patel D, Kim ES, Hsiai TK. *Microelectromechanical Systems, Journal of.* 2008; 17(5):1178–1186.
- Zhang BX. *International Journal of Numerical Methods for Heat & Fluid Flow.* 2009; 19(5):561–573.
- Zilla P, Moodley L, Scherman J, Krynauw H, Kortsmit J, Human P, Wolf MF, Franz T. *Journal of Vascular Surgery.* 2012; 55(6):1734–1741. [PubMed: 22386141]

Highlights

We combined multi-modality to assess plaque development in fat-fed rabbit aorta.

In vivo MEMS thermal sensor deployment revealed higher intravascular shear stress.

Impedance sensor correlated lipid content with elevated electrochemical impedance.

Elevated impedance in plaques was consistent with ultrasound imaging and histology.

Three-modality approach improves lipid detection in unstable atherosclerotic plaque.

Figure 1. Catheter-based concentric bipolar microelectrodes

(a) Magnification of concentric bipolar microelectrodes. **(b)** Packaging of the concentric bipolar microelectrodes to the coaxial wire.

Figure 2. Comparison of intravascular shear stress profiles with Computational Fluid Dynamics (CFD) simulations

Fluoroscopic guidance allowed for positioning the catheter at the (a)–(d) distal aortic arch, (e)–(h) thoracic aorta, (i)–(l) peri-renal abdominal aorta, and (m)–(p) infra-renal aorta. The thermal sensor was able to capture the characteristic pulsatile profiles along the aorta with a high spatial and temporal resolution. The red, solid curves denoted ISS obtained by the sensors, whereas the blue dashed curves denoted the CFD simulations.

Figure 3. ISS profiles in response to normal diet

(a) Representative baseline ISS profiles obtained from the abdominal aorta of rabbits. (b) Representative ISS profiles obtained from the same region after 9 weeks of normal chow diet (ND). The solid lines represented averaged shear stress over 10 cardiac cycles; dashed lines represented average ISS $\pm$ standard deviation. (c) Time-average ISS profiles in the distal aortic arch, thoracic aorta, abdominal aorta and infra-renal aorta regions were compared between baseline and after 9 weeks of ND. No statistically significant differences in ISS profiles were observed. (d) Representative cross-section of the thoracic aorta by IVUS imaging and histology revealed absence of endoluminal lesions on ND.

Figure 4. ISS profiles in response to high-fat diet

(a) Representative baseline ISS profiles obtained from the abdominal aorta of rabbits. (b) Representative ISS measured obtained from the abdominal aorta of rabbits on high-fat diet (HD) for 9 weeks. The solid lines represented averaged shear stress over 10 cardiac cycles; dashed lines represented average ISS $\pm$ standard deviation. (c) Time-average ISS profiles in the distal aortic arch, thoracic aorta, abdominal aorta and renal aorta regions were compared between baseline and after 9 weeks of HD. * denoted statistically significant differences in time-averaged ISS signals at baseline and after 9 weeks of HD ($P < 0.05$). (d) Representative cross-section by IVUS imaging and histology of the thoracic aorta segment. Positive staining (reddish-brown) indicated presence of oxLDL. The discontinuations of the endoluminal lesions at 10 and 12 o'clock were due to branching.

Hematological analysis

Figure 5. LDL and blood viscosity at baseline versus after 9 weeks

(a) LDL levels significantly increased after 9 weeks in rabbits on high-fat diet. **(b)**

Kinematic viscosity was significantly higher in rabbits on high-fat than on normal diets after 9 weeks.

Figure 6. Endoluminal EIS assessment of oxLDL-rich lesions

(a) Frequency-dependent impedance was measured from 10kHz to 100kHz (Logarithm scale). (b) Phase angles were measured from the corresponding frequency range (Logarithm scale). (c) Equivalent circuit model to simulate tissue resistance provided validation of the experimental EIS signals as previously described (Yu et al. 2011b). CPE: constant phase element. (d) The representative IVUS image revealed the endoluminal lesions at 4 o'clock. The corresponding histology confirmed the pre-atherosclerotic lesions. (e) Immunohistochemistry revealed prominent anti-oxLDL staining (reddish-brown). (f) The bar graph compared the simulated tissue resistance between normal ($n = 8$) and oxLDL-rich lesions ($n = 12$) (* $p < 0.05$).