Journal of Biomechanics 50 (2017) 11–19

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Spatial phenotyping of the endocardial endothelium as a function of intracardiac hemodynamic shear stress

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ARTICLE INFO

Article history:
Accepted 2 November 2016

Keywords:
Endocardial endothelium
4D Flow MRI
Heart
Shear stress
RNA sequencing

ABSTRACT

Despite substantial evidence for the central role of hemodynamic shear stress in the functional integrity of vascular endothelial cells, hemodynamic and molecular regulation of the endocardial endothelium lining the heart chambers remains understudied. We propose that regional differences in intracardiac hemodynamics influence differential endocardial gene expression leading to phenotypic heterogeneity of this cell layer. Measurement of intracardiac hemodynamics was performed using 4-dimensional flow MRI in healthy humans ($n=8$) and pigs ($n=5$). Local wall shear stress (WSS) and oscillatory shear indices (OSI) were calculated in three distinct regions of the LV – base, mid-ventricle (midV), and apex. In both the humans and pigs, WSS values were significantly lower in the apex and midV relative to the base. Additionally, both the apex and midV had greater oscillatory shear indices (OSI) than the base. To investigate regional phenotype, endocardial endothelial cells (EEC) were isolated from an additional 8 pigs and RNA sequencing was performed. A false discovery rate of 0.10 identified 1051 differentially expressed genes between the base and apex, and 321 between base and midV. Pathway analyses revealed apical upregulation of genes associated with translation initiation. Furthermore, tissue factor pathway inhibitor (TFPI; mean 50-fold) and prostacyclin synthase (PTGIS; 5-fold), genes prominently associated with antithrombotic protection, were consistently upregulated in LV apex. These spatio-temporal WSS values in defined regions of the left ventricle link local hemodynamics to regional heterogeneity in endocardial gene expression.

1. Introduction

Endothelial cell (EC) structure and function is strongly influenced by the frictional force of wall shear stress (WSS) at the blood-EC interface. The effects of WSS on EC phenotype are well described to play an important role in blood vessel physiology and pathology. In regions where undisturbed WSS dominates, ECs are healthy. Conversely, ECs in regions of disturbed WSS, characterized by flow separation and transient flow reversals, have a pro-inflammatory, pro-oxidative stress phenotype and represent sites where atherosclerosis preferentially develops (Civelek et al., 2009; Davies et al., 2013).

Endocardial endothelial cells (EECs) line the heart chambers and represent an important barrier between the circulation and underlying myocardium. Studies in rat and pig demonstrate that EECs, which may originate from precursor vascular ECs, differ from mature vascular ECs with regard to morphology, gene and protein expression and labile molecule production (Hendrickx et al., 2004; Mebazaa et al., 1995). EECs are important in regulating cardiac contraction; endocardial denudation results in loss of contractile strength (Brutsaert et al., 1988; Mebazaa et al., 1993; Shen et al., 2013; Smith et al., 1991). This is attributed to the production and release of paracrine signaling agents such as nitric oxide (NO)

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http://dx.doi.org/10.1016/j.jbiomech.2016.11.018
0021-9290/

(Smith et al., 1991). Unlike vascular ECs, the role of hemodynamics in the regulation of EEC biology has not been carefully studied.

Preservation of flow patterns throughout the LV is important to maintain its efficient function (Gharib et al., 2006). For example, diastolic vortex formation prevents the dissipation of blood kinetic energy and therefore reduces the myocardial force required to eject blood during systole (Kilner et al., 2000). Recent advances in cardiac imaging have enabled more detailed descriptions of intracardiac hemodynamics (Rodriguez Muñoz et al., 2012). In particular, the development of four-dimensional (4D) flow MRI enables 3D velocity encoding with ECG-gating, providing accurate spatial and temporal information. This technology has demonstrated that flow patterns within the LV change with age and gender and that blood residence times vary within the LV (Föll et al., 2013; Hendabadi et al., 2013). The influence of spatio-temporal differences of hemodynamics on EEC biology in the LV is poorly understood.

Here we calculated regional LV WSS using 4D flow MRI in humans and pigs to show that WSS varies regionally throughout the left ventricle of both species. We then isolated EECs from sites matched to the regional hemodynamic characteristics in pig LV and profiled gene expression by RNA sequencing to demonstrate significant regional differences of EEC phenotypes.

2. Methods

2.1. 4D Flow MRI acquisition

Four-dimensional (4D) flow MRI (4D flow MRI) was performed to assess intracardiac hemodynamics in humans and pigs. For human MRI, IRB approval and informed consent were obtained from all 4D flow MRI study participants. The specifics of image acquisition and analysis were as previously described (Markl et al., 2012, 2016; McCormick et al., 2016). Studies were performed on 8 healthy volunteers (mean age: 24 ± 1.8 years; four females and four males, heart rate 63.5 ± beats per minute, LV ejection fraction (EF) % 65.3 ± 2.1). Animal experiments were performed in compliance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (NIH publication 85–23, revised 1996) and approved by the University of Pennsylvania Institutional Animal Care and Use Committee. For MRI analysis, healthy adult pigs ($n=5$, Yorkshire swine, weight = 61.6 ± 2.9 kg; range: 59–67 kg) were used. 4D flow MRI was performed with a dual cardiac and respiratory prospectively-gated cine phase-contrast MRI sequence with the following parameters: temporal resolution = 20.8 ms, spatial resolution = 2 × 2 × 2 mm^3, flip angle = 8°, field of view = 320 mm × 320 mm, pixel bandwidth 460 Hz/pixel. The velocity encoding (V_{enc}) sensitivity was adjusted for each animal to minimize velocity aliasing during diastole (V_{enc} = 75–185 cm/s) as previously described (Witschey et al., 2015).

2.2. Endocardial endothelial cell sample isolation and RNA sequencing

Endocardial endothelial cells (EEC) were isolated from 8 pigs. Incisions were made 5 mm from the apex of the heart to expose the apical endothelium. A longitudinal incision was made 0.5–1 cm left of the left anterior descending (LAD) artery to expose the main LV chamber where base and midV EEC samples were collected. Approximately 1–2 cm^2 of endothelium were gently scraped from each region (Fig. 5A) and placed in RNA isolation buffer (mirVana Isolation Kit, ThermoFisher, Waltham, MA). Nucleic acid isolation, purification, QC and concentration were performed as previously described (Jiang et al., 2015). Pure populations of EECs were confirmed by Western blot and immunofluorescence.

RNA samples from base, midV and apex in 8 male pigs were submitted to the Children's Hospital of Philadelphia High-Throughput Sequencing Center for quality control, library construction and sequencing using the Illumina HiSeq 2500 with 100 base pair paired-end reads and yielding an average of about 32 million read pairs per sample. Reads were aligned to the Sscrofa10.2.73 genome with STAR (Dobin et al., 2013) and the PORT pipeline (https://github.com/itmat/Normalization) was used for normalization and quantification. Differential gene expression (with comparisons run in paired-by-pig mode) was performed using edgeR (Robinson et al., 2010) and PADE (https://github.com/itmat/pade), an extension of PaGE (Grant et al., 2005). For analysis, we used genes identified by both approaches at FDR ≤ 0.10. Additionally, genes with average normalized counts less than 50 in each of the two regions compared were excluded from analysis.

2.3. Pathway analysis

Data were analyzed by QIAGEN Ingenuity® Pathway Analysis software (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity). Gene Ontology (GO) enrichment analysis (GO Biological Process and Molecular Function) was performed using the DAVID (Database for Annotation, Visualization and Integrated Discovery) web

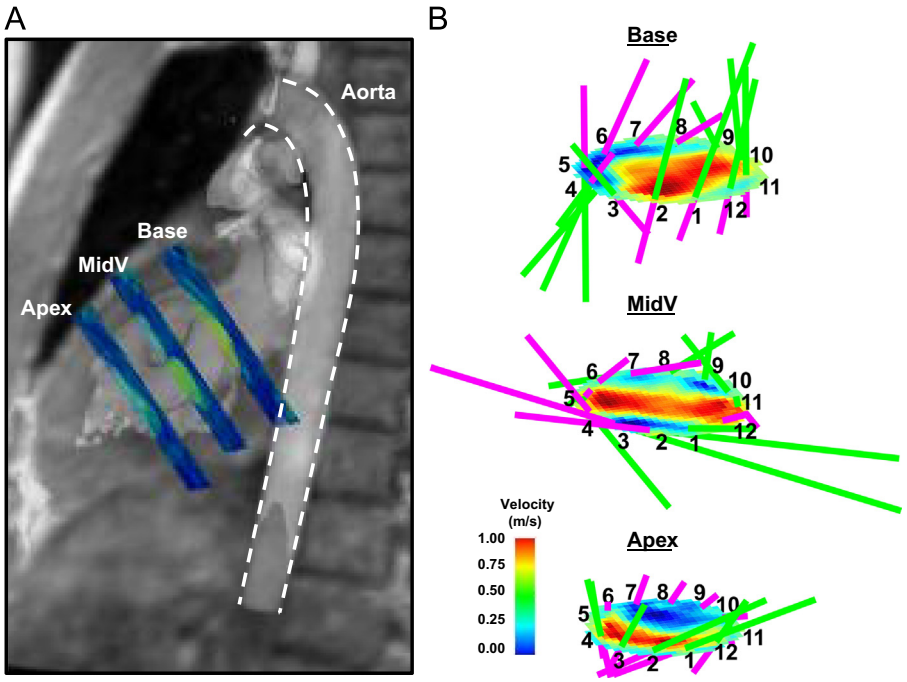

Fig. 1. Analysis of 4D Flow MRI. A, Representative human 4D flow MRI image showing base, midV, and apical two-dimensional plane selection. Planes are color coded for velocity. B, Representative results of time-averaged WSS_{mag} and OSI calculations for base, midV and apex. Following manual segmentation of the LV luminal area, WSS and OSI values were extracted from intraventricular velocities for 12 discrete points. Luminal colors indicate intraventricular blood flow velocities (m/s). The green lines emanating from the 12 points indicate WSS magnitude (line length) and direction. Purple lines show OSI magnitude only (line length) at the same 12 points and each is linked to the same position (1–12) as the WSS data (i.e. there is no directional information in the OSS data). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)