Medical Bio Trial Use Proposal: Medical Bio EX Proposal Report

Proposal Number: 2008B1969

Title: Phase-contrast imaging of blood flow and shear in mouse aortic disease models

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Beamline Used: BL20XU

Research Purpose and Background:

Aims: This study aims to develop a new quantitative measure of in-vivo blood flow using the penetrating and high intrinsic contrast afforded by Phase Contrast X-ray Imaging coupled with the accurate and quantitative fluid dynamics information provided by Particle Image Veclocimetry (PIV). The ability to image flow using such a technique would find widespread application in the study of vascular disease. This study has two desired outcomes:

1. Application of the method to achieve significant measurements of both morphology and function of baseline (C57BL/6) and transgenic (apoE -/-) ex-vivo vasculatures.
2. Development of the experimental methodology towards physiologically meaningful flow rates and in-vivo capability;

Atherosclerosis is the leading cause of death in the developed world and nearly the leading cause in the developing world. It is associated with systemic risk factors including hypertension, smoking, hyperlipidemia and diabetes mellitus. Nonetheless, atherosclerosis remains a geometrically focal disease, preferentially affecting the outer edges of vessel bifurcations. In these predisposed areas, hemodynamic shear stress, the frictional force acting on endothelial cell surface as a result of blood flow, is weaker than in protected regions.

In-vitro and low-resolution ultrasound imaging studies have identified hemodynamic shear stress as an important determinant of endothelial function and phenotype. The functional regulation of the endothelium by local hemodynamic shear stress provides a model for understanding the focal propensity of atherosclerosis in the setting of systemic factors and may help guide future therapeutic strategies.

The relation between shear stress and atherosclerosis is based almost exclusively on low resolution observational studies in humans and large animals. In-vitro data have shown that subtle shear stress changes can modulate the response of cultured endothelial cells, leukocyte adhesion and thrombus formation, which has been shown to mediate atherogenesis and the final stages atherosclerotic plaque rupture (stroke, myocardial infarction etc), respectively.

To date, the in-vivo data on flow velocimetry are limited to thin walled vasculature. More specifically, high-resolution near-wall fluorescent micro particle image velocimetry has been used in mouse cremaster muscle venules. Whilst these studies provide information on flow velocities associated with cell adhesion in the microvasculature, they do not provide insight into the role of shear stress and atherosclerosis in resistant vessels. To provide such evidence, an appropriate in-vivo model that can generate complex shear stress fields is required. To calculate shear stress accurately, very high resolution measurements of velocity are required (Fouras and Soria 1998). Other measurement modalities such as MRI and ultrasonography are incapable of the required resolution.

Recent studies (Fouras et al., 2007, Lee and Kim 2006,) have demonstrated the remarkable potential of X-ray phase contrast imaging to provide high resolution in-vitro blood flow field measurements by use of Particle Image Velocimetry (PIV), an optical technique routinely used by engineers, for example in aerodynamics. Explained in its most simple form, PIV is an image processing technique which uses statistical (by use of correlation functions) inter-frame comparisons of sub-regions of images to determine motion between those frames in all of those sub-regions. Figure 1 shows a 3D velocity vector field of the flow within a tube measured by use of the single projection PIV algorithm described in Fouras et al., 2007.

Using the penetrating ability of X-rays, we can measure flows inside blood vessels (see Fig. 2) but our pilot studies have revealed that there is a need for very high flux (and low exposure times) to accurately characterize physiological flows. Using X-ray phase contrast, the blood cells themselves provide the seed particles for tracking flow, eliminating the need for tracking particles or other contrast agents. The very high speed, time resolved, phase contrast images that are uniquely available on BL20XU will allow PIV flow measurements of the vascular flow at physiologically relevant flow rates.

Aims:

This proposal covers 2 sets of experiments with separate aims:

1. We will begin with PIV measurements of blood pumped through custom manufactured in-vitro flow models (n=4). The blood will at be pumped in both a continuous and pulsatile (mimicking the cardiac cycle) fashion. Finally a new kind of measurement Computed Tomographic PIV will be trialled on the in-vitro models.

2. We have developed an ex-vivo flow arterial model that allows physiological in-vivo aortic conditions to be simulated. Dissected mouse aorta (n=18) will be mounted between graduated glass capillaries within a custom designed flow device/tissue bath. This device allows the perfusion of anti-coagulated blood through the aorta via an infusion pump, while maintaining the aorta under physiological conditions.

Experimental/Analytical Method:

Experiment 1 consists of a number of in-vitro experiments. These experiments start with blood (both with and without contrast agent) pumped through in-vitro models (1 straight, 1 stenosed, 1 curved and 1 bifurcating

tube). Blood will be pumped at flow rates which are physiologically similar to the aortic flow of a mouse. The blood will at first be pumped in a continuous fashion, but then a pump, which mimics the action of the heart (Harvard apparatus pulsatile blood pump), will be used. Finally, a fully 3D measurement technique Computed Tomographic PIV will be employed by scanning the sample from a small number (10-19) of different angles.

Experiment 2: We have developed an ex-vivo flow arterial model that allows physiological in-vivo aortic conditions to be simulated. Wildtype and transgenic mice (C57BL/6, n=4 & apoE-/-, n=14), are euthanised and the aorta exposed via a midline incision through the abdomen, and retraction of the intestines. The aorta is dissected from the vena cava for a distance of 2-3 mm immediately inferior of the renal arteries. The dissected aorta is mounted between graduated glass capillaries within a custom designed flow device/tissue bath. This device allows the perfusion of blood through the aorta via a pump, while maintaining the aorta under physiological conditions. Base-line studies will be conducted using tissue from C57BL/6 mice, measuring not only the structural detail of the aorta but also the flow and shear within the vessel. Comparisons will be made between the C57BL/6 and apoE-/- mice which are expected to contain a number of atherosclerotic plaques (after high cholesterol diet).

Research Results:

Lost Beam Time

Due to equipment failure, a large part of the beam time was lost, so the most exciting experiments involving animals could not be performed and the rest of the experiments were rushed.

However , we have been successful in the evaluation of the technique to quantify blood flow at physiological flow rates in-vitro. Validation of imaging data results from the known flow rates applied by the pump.

Status of Publication and Patent:

1. **Dubsky, S.**, **Jamison, R.A.**, Irvine, S.C., Siu, K.K.W., **Hourigan, K.** & **Fouras, A.** (2009) Computed tomographic X-ray velocimetry. Submitted to Applied Physics Letters.

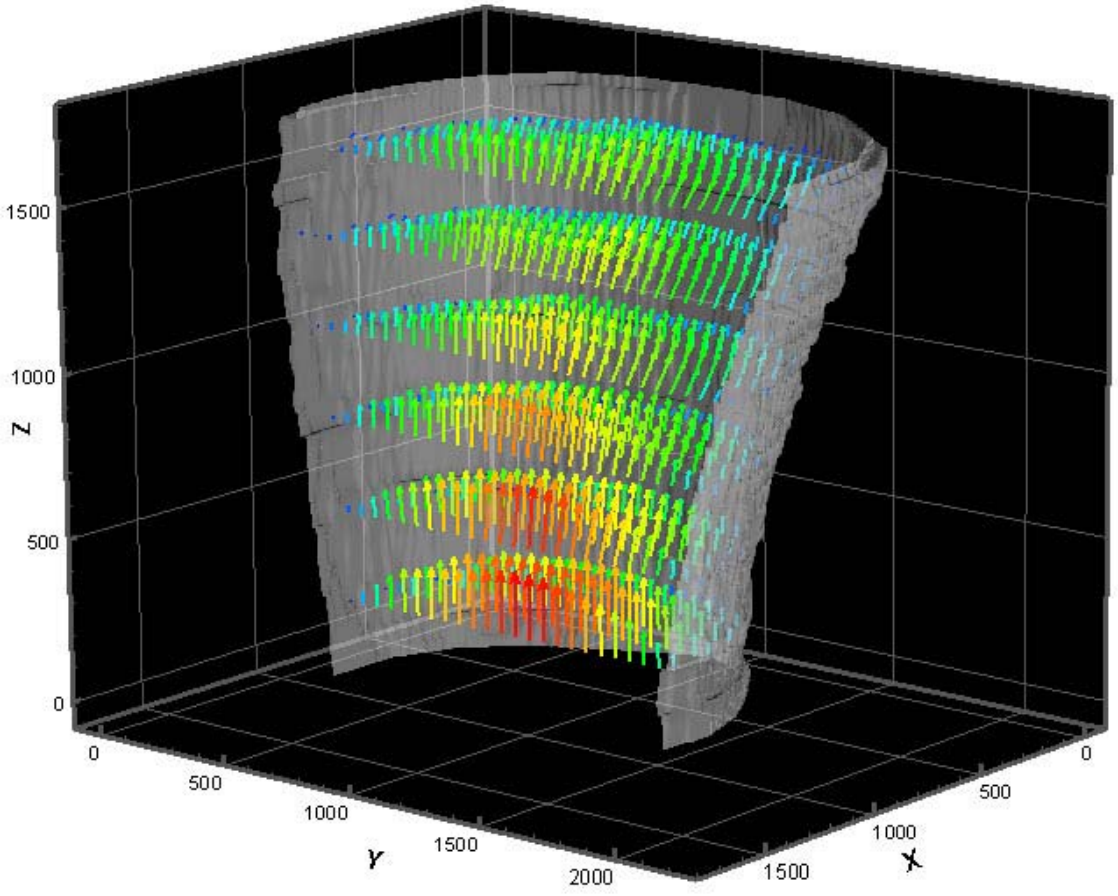

Figure 1. Successful 3D measurement of blood flow in in-vitro model of vasculature