BLOOD FLOW CHARACTERIZATION IN A PERFUSED COLLAGEN VESSEL BIOREACTOR USING X-RAY MICRO-PIV

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Abstract

The design of well-characterized, adaptable, blood-perfused tissue models has become a central objective of the emerging field of tumor engineering, as physiologically accurate \textit{in vitro} vascular models can provide new avenues for modulation of the tumor microenvironment and preclinical evaluation of the therapeutic potential of new treatments. The characterization of fluid parameters in perfused tissue models is a critical step towards understanding and manipulation of the tumor microenvironment. The recent development of x-ray phase contrast particle image velocimetry has enabled measurement of blood flow in simple systems. This study presents high-resolution blood velocity measurements in a three-dimensional collagen microvessel bioreactor using synchrotron x-ray illumination. These results provide the groundwork for future flow measurements in a fully functioning blood-perfused tumor model.

Introduction

Newly developed cancer therapies must pass through a series of increasingly complex testing regimens before obtaining FDA approval as valid treatments. The costs of these tests increase rapidly as the physiological accuracy of the platform increases, from initial proof-of-concept in static tissue cultures, to validation in animal models, and ultimately to multi-phase human clinical trials. Of the therapies that enter clinical trials, the most expensive stage of development, only 5\% obtain approval by regulatory authorities (Kola and Landis 2004). With the average cost of development of a single drug above $800 million (DiMasi, Hansen et al. 2003), the oncologic community needs more robust pre-clinical evaluation methods for better assessment of the potential of new therapies (Humphrey, Brockway-Lunardi et al. 2011).

Three-dimensional engineered microfluidic tumor models are becoming increasingly important as intermediate platforms for the development of cancer therapies. 3D tumor models are superior to two-dimensional cultures in their reproduction of relevant physiological conditions and are inexpensive in comparison to animal models and clinical trials (Ingram, Techy et al. 2010; Stroock and Fischbach 2010). Because fluid shear stress is known to play a pivotal role in tumor development processes such as tumor cell expansion, angiogenesis and metastasis (Verbridge, Chandler et al. 2010), perfused vascular models are superior to static cultures. Our group has developed a novel 3D perfused vessel bioreactor that supports the growth of tumor cells within a collagen scaffold and endothelial cells on the lumen of the microchannel (not yet published). The collagen hydrogel exhibits mechanical and chemical stability while supporting cellular growth, organization, and structural remodeling similar to that found \textit{in vivo} (Chrobak, Potter et al. 2006). This model provides a basic platform to study interactions between tumor and endothelial cells under a
range of microfluidic conditions, as well as the effects of novel cancer therapies on tumor-endothelial cross-talk and vascular sprouting. Initial studies using this tumor model have provided new insight into tumor-endothelial cross-talk under dynamic conditions. To increase the fidelity of the collagen vascular tumor model, it can be perfused with whole blood rather than cell culture media to better reproduce the in vivo vasculature.

Characterization of the flow within three-dimensional tumor models is critical for quantifying fluid shear stress and fluid-cell interactions to build a physiologically accurate platform for drug evaluation. We have successfully measured the flow of transparent cell culture media within an acellular collagen vessel bioreactor using particle image velocimetry (PIV), a noninvasive optical flow measurement technique, and found that it closely matches the Poiseuille profile expected for low-Reynolds number flow. PIV is a well-established technique in which fluorescent tracer particles seeded within a flow of interest are illuminated by a laser sheet and imaged with a high-speed camera; subsequently, image pairs are correlated to calculate a velocity field with high spatio-temporal resolution. We have also demonstrated that PIV can be used to quantify flow conditions within a transparent cellularized bioreactor (Voigt, Buchanan et al.). Unfortunately, because PIV requires optical access to the region of interest, the use of standard techniques is limited to thin-walled tissues perfused with a transparent fluid. For 3D bioreactors perfused with blood, which is optically opaque due to the hemoglobin present in red blood cells, standard PIV techniques cannot be used.

X-ray PIV is a recently developed variation of standard optical PIV that permits measurement of flow within opaque vessels. In this technique, phase contrast imaging of x-ray illuminated particles in the fluid volume replaces optical imaging of laser-induced fluorescent particles. X-ray PIV has been successfully demonstrated for measurement of blood flow within glass channels and rigid stenosis models (Lee and Kim 2005; Kim and Lee 2006; Jamison, Dubsky et al. 2011). These results demonstrate the high potential of x-ray PIV as a technique for characterization of in vitro and in vivo flows; however, no x-ray PIV has previously been performed to measure flow in tissues.

In this work, we examine the feasibility of accurate flow field measurements in blood-perfused collagen microvessel tumor models for in vitro drug evaluation. We validate the accuracy of PIV analysis for data acquired in a collagen vessel bioreactor under acellular conditions using x-ray phase contrast imaging.

**Materials and Methods**

**Experimental Cases**

Three sets of experimental conditions were considered: a 742 µm collagen vessel perfused with whole bovine blood and two rigid polymer vessels with lower complexity for comparison: 756 µm diameter polytetrafluoroethylene (PTFE) perfused with a glycerine solution, and 867 µm diameter fluorinated ethylene propylene (FEP) perfused with whole bovine blood. Collagen is used because it comprises the majority of tumor extracellular matrix. PTFE and FEP are interchangeable flexible transparent plastics that are non-reactive with blood. The glycerine solution was used because it matches the majority of tumor extracellular matrix. PTFE and FEP are interchangeable flexible transparent plastics that are non-reactive with blood. The glycerine solution was used because it matches the viscosity of blood and could therefore be perfused at the same rate as blood to achieve the same Reynolds number. It also matches the density of blood, meaning that particles neutrally buoyant in blood were also neutrally buoyant in the glycerine solution. The design and manufacture of the collagen
microchannel are as follows: briefly, type I collagen stock solution was prepared from rat tail tendons dissolved in pH 2.0 HCl and combined with 10X Dulbecco’s Modified Eagle Medium (DMEM), 1N NaOH and deionized water to prepare a solution with 10 mg/mL collagen. The collagen solution was injected into a 5 cm long 3 mm diameter FEP shell fitted concentrically with a 5 cm long 22 gauge (711 μm) stainless steel needle and sealed at the ends with acrylic sleeves. The solution was cured for 30 minutes at 37°C, after which a cylindrical microchannel was created in the collagen hydrogel by removal of the needle. 1.27 cm long stainless steel needles were inserted into the acrylic sleeves at the ends of the collagen vessel and into the ends of the PTFE and FEP vessels to provide luer interfaces for connection with the flow system. Figure 1a depicts the collagen microchannel (not to scale).

Working Solutions

Two working solutions were used for these experiments. A 50% v/v solution of water and glycerine was perfused in the PTFE channel as a control. Fresh whole bovine blood (Bovine Blood in Citrate Anticoagulant, Quad Five) was perfused in the FEP and collagen channels. Experiments were performed within 48 hours of the time that blood was drawn, and all procedures involving blood were performed under sterile conditions and using equipment washed with phosphate buffer solution (PBS). Blood was kept refrigerated until shortly before experiments were performed, at which time it was heated to room temperature (22°C). The dynamic viscosity of whole blood was measured to be 8.15 cP at 21.5°C and the viscosity of the 50% v/v water/glycerine solution was measured to be 9.5 cP at 22.5°C. The density of whole blood is nearly that of water (1.0 g/cm³), while that of a 50% v/v water/glycerine solution at 22°C is 1.1 g/cm³.

To provide phase contrast for PIV, both the blood and glycerine solution were seeded with hollow glass microspheres (Sphericel, Potters Industries) with 10 μm nominal diameter. Because bulk Sphericel microspheres have wide diameter and effective density distribution (2-20 μm diameter), neutrally buoyant particles were isolated as follows: Dry glass microspheres were suspended at a high concentration in distilled water and allowed to rest for 24 hours, during which time they separated into dense sediment, a suspension layer, and a layer of floating particles. The central suspension layer was pumped through a 10 μm filter (Whatman) into a clean flask and heated to evaporate excess water and obtain a high concentration of neutrally buoyant microspheres.

Because the negative surface charge acquired by glass immersed in water can activate the platelets in whole blood and initiate coagulation, it was necessary to first treat the glass microspheres with blood plasma to provide a protective protein coating on the glass surfaces before making a suspension of whole blood and particles. First, the filtered glass microsphere suspension was boiled until...
dry. The remaining dry microspheres were scraped from the flask and collected. Whole blood was centrifuged and plasma supernatant was extracted. 220 mg of dry filtered particles were suspended in 1 mL of plasma and incubated for 24 hours at 37° C. Finally, 5 mL whole blood was added to the plasma solution for the final working solution: blood seeded with 37 mg/mL neutrally buoyant hollow glass microspheres.

For the water/glycerine working fluid, the highly concentrated filtered particle solution was diluted with distilled water to approximately twice the concentration of the whole blood solution and mixed with an equal volume of glycerine to obtain the second final working solution: 50% v/v water/glycerine seeded with approximately 40 mg/mL neutrally buoyant hollow glass microspheres.

Experimental Procedures

A syringe was filled with the working solution for each experimental condition and loaded onto a syringe pump (Harvard Apparatus, PhD). 61 cm of flexible PVC tubing with luer ends was used to connect the syringe and vessel inlet needle and to connect the vessel outlet needle with a free-surface collection reservoir. Prior to flow measurements, the vessel was primed with the working fluid and flushed for several seconds at 5 mL/min to clear any adhered or aggregated particles from the vessel. During flow measurements, the fluid was perfused steadily through the vessel at a flow rate of 5 µL/min. The Reynolds number was 0.03 for all experimental cases.

X-Ray Imaging

X-ray particle images were acquired at the X-ray Operations and Research beamline 32-ID-B of the Advanced Photon Source, Argonne National Laboratory. For each experiment, the vessel (PTFE, FEP, or collagen microvessel) was fixed in the path of a monochromatic x-ray beam with 1 mm² cross-sectional area. The beam was converted to visible light by a scintillator crystal and magnified with a 5x objective lens for imaging. 1.2 MP images were recorded at 15, 20, or 30 Hz with a machine-vision camera (AVT Pike F-505B) (Figure 1b). The image resolution was found to be 1.32 µm/pixel using a 400-mesh gold TEM grid as a reference. A minimum of 2000 images was collected for each experimental case.

Data Analysis

Mean subtraction and a high-pass Gaussian filter in the Fourier domain were applied to the images to remove the background and equalize illumination for enhanced correlation accuracy (Figure 2a, b). In Figure 2a, there is little contrast between particles and background. In Figure 2b, the particles are much more easily distinguished from the background. Additionally, a mask was applied to the images to remove inclusions such as bubbles in the collagen before performing correlation (Figure 2c). Images were correlated using robust phase correlation as described in (Eckstein, Charonko et al. 2008; Eckstein and Vlachos 2009; Eckstein and Vlachos 2009) with a correlation particle diameter of 10 pixels and using a single-pass ensemble algorithm (Meinhart, Wereley et al. 2000) with window deformation (Scarano 2002) over a minimum of 2000 image pairs. Three iterations of window deformation were applied. Between iterations, correlations were validated using a median universal outlier detection scheme (Westerweel and Scarano 2005) and smoothed using a Gaussian filter. Correlation windows of 4 x 64 pixels with 75% overlap were used, resulting in a spatial vector resolution of 1.32 µm in the axial direction and 21 µm in the radial direction.
The mean velocity field along the length of the vessel was computed for the resulting vector fields and used for computation of the mean WSS using the thin-plate spline radial basis functions as described in (Karri, Charonko et al. 2009).

**Results**

Recorded images were successfully correlated and velocity fields were computed. Figure 3 shows vector plots of the ensemble velocity field obtained for each data set (PTFE perfused with glycerine, FEP perfused with blood, and collagen perfused with blood), which demonstrate significant differences between test cases. It should be noted that, because the image signal-to-noise ratio (SNR) was extremely low (Figure 2a, b), the resulting velocity fields were noisy. Additionally, because of masking applied at inclusions of air bubbles within the collagen hydrogels, velocity information was not available in several regions of each data set (Figure 2c). Loss of correlation at the vessel ends, which causes many of the invalid vectors seen in Figure 3, is due to particles entering and exiting the field of view at the top and bottom of the images as well as poor illumination in those image regions (dark areas in Figure 2a). Despite the poor image quality and highly noisy correlations, important conclusions can be drawn from the data.
To validate the results, the mean velocity profiles were computed and compared with theoretical models. Newtonian fluid perfused steadily in a rigid tube exhibits a parabolic Poiseuille profile; however, blood flow at low Reynolds number is predicted to exhibit a blunt velocity profile due to its non-Newtonian nature. The is due to the Fåhræus–Lindqvist effect, in which the aggregation of red blood cells in the center of the vessel leaves a cell-free plasma layer with lower viscosity at the vessel walls (Ku 1997). The resulting profile can be estimated using a cell-free marginal layer model (Chandran, Rittgers et al. 2007).

Figure 4 compares the volumetric mean velocity profile in the microvessel, obtained from PIV analysis, with the Poiseuille parabolic profile and the cell-free marginal layer model for blood. It can be seen that neither the marginal layer model nor the Poiseuille model closely predicts the behavior of the blood flow. This is reflected in the mean WSS, which is estimated to be 0.0028 dyn/cm$^2$ for the PTFE vessel, 0.066 dyn/cm$^2$ for the FEP vessel, and 0.077 dyn/cm$^2$ in the collagen channel based on the volumetric data obtained using PIV. These values are significantly lower than the WSS predicted by the Poiseuille solution and the marginal layer model: 0.78 dyn/cm$^2$ for the PTFE vessel, 0.71 dyn/cm$^2$ for the FEP vessel, and 1.13 dyn/cm$^2$ for the collagen channel. The difference in theoretical values for the channels is based on the diameter variation.

The sudden drop-off of velocity magnitude and resulting low WSS in the PTFE vessel data is indicative of loss of correlation in the vessel. This effect was apparent in all vessels when standard processing was used. Optimization of PIV processing succeeded, allowing more accurate velocity information to be extracted from the data; however, the PTFE case still requires further optimization to achieve maximum correlation of the true flow present in the vessel.
Figure 4: Velocity profiles comparing data with theory. Top left: PTFE vessel. Top right: FEP vessel. Bottom: Collagen channel. Cross-sectional (dashed) and volumetric (solid) profiles are plotted for theoretical Poiseuille (red) and marginal layer model (green). Volumetric PIV data (blue) is plotted with the 95% CI on the mean (dashed).

We hypothesize that the PTFE case exhibits the poorest correlation characteristics because, contrary to the other two cases, no red blood cells were present in the flow which can contribute to correlation strength.

It is noteworthy that the velocity profiles obtained from the data exhibit bias towards one side of the vessel, a phenomenon particularly significant in the FEP vessel. This is an experimental artifact due to vessel curvature.

Conclusions and Future Work

This work demonstrates a new capability for x-ray PIV in a blood-perfused collagen vessel bioreactor for quantification of flow conditions and wall shear stresses, permitting future investigation of flow conditions within tumor models for a more complete understanding of cellular response to fluid conditions. We have shown that the velocity profile of blood flow in a collagen microchannel does not match theoretical models. These results indicate the necessity of flow measurements in tumor models for accurate estimation of fluid shear stresses experienced by the tumor endothelium.

The data indicate that further improvement of PIV techniques to better correlate data with large, heterogeneous particle distribution and low SNR can significantly increase correlation accuracy. Optimization of PIV validation will also be performed for better identification of inaccurate measurements.
In conclusion, the results presented here outline a method for high-resolution flow measurements in opaque in vitro microvascular tumor models, paving the way for future work in analysis and control of tumor microvasculature.

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References


