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Microvascular Branching as a Determinant of Blood Flow by Intravital Particle Imaging Velocimetry

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ABSTRACT
The effects of microvascular branching on blood flow were investigated in vivo by microscopic particle image velocimetry (micro-PIV). We use micro-PIV to measure blood flow by tracking red blood cells (RBCs) as the moving particles. Velocity flow fields, including flow pulsatility, were analyzed for the first four branching orders of capillaries, postcapillary venules, and small vessels of the microvascular network within the developing avian yolk sac at embryonic day 5 (E5). Increasing volumetric flowrates were obtained from the flow profiles as a function of increasing vessel diameter and branching order. Maximum flow velocity increased approximately two-fold with increasing vessel order and branching order, reaching a maximum of 100–150 μm/s in the capillaries. Our study will be useful for advancing the understanding of blood flow within anastomotic, heterogeneous microvascular networks. (Supported by NASA Glenn Research Center RC04-54.)

INTRODUCTION
It is hypothesized that the dynamics of blood flow, including velocity, pressure and shear stress, are influential factors in microvascular remodeling and angiogenesis. Blood flow is primarily laminar and therefore by Hagen–Poiseuille's law, blood flow should be inversely proportional to the fourth power of vessel radius (r). Our research focuses on the role of vessel morphology in microvascular remodeling, angiogenesis and lymphangiogenesis. We quantify vascular remodeling as a function of vessel morphology, diameter, branching order, and position by using the NASA Glenn computer software VEGAS.14 For this study, we are investigating blood flow as a function of vessel diameter by intravital particle imaging velocimetry (PIV).1 PIV measures local velocities within a flow field from image-to-image cross-correlation of particle displacement. We utilize this optically accessible micro-PIV of the avian embryonic yolk sac. Red blood cells (RBCs) serve as the moving particles, so that fluorescent tracking particles are not required.

METHODS
Intravital Imaging and Post-Processing. Fetalisized eggs were cultured in coveit, oval-Petri dishes (Column: Cox: jackson) were cultured between 37.5°C and 32°C. All embryonic day 5 (E5), a field of view was selected from the yolk sacs, velocitography (Fig. 1A) for the imaging of four successive vessel branching orders (E5-B1-B4) that included successive vascular branching generations and capillary venules (Fig. 1B). The Petri dish was placed on a microscope stage, warmed to 37°C and then under an infrared light trap. A digital image stack (Fig. 1C) was acquired by 256 images (image size 1024 x 1024 pixels at 125 frames per second) at vessel spacing was acquired in propap (250 levels) at a total magnification of 14× (resolution = 0.07 μm/pixel) with an Olympus BXZ2 stereo microscope and a high-speed, high-magnification Photonic Image Company MPPS digital camera and Photonic FastCompare PIV (FC-PIV) software, illuminated by a 100W microscope lamp with a 9U filter. A set of four-image stacks, B1-B4, was acquired from each of the embryos. Each representative image set was reported here. Images were processed by subtracting each image from itself to provide a clear image obtained by analyzing the image stack with block and vessel tracking software in PIV analysis. Noise was reduced by adjusting the adjuster, a single pixel tracking pixel only displaying the vessel field was selected by scanning the aligned images as an integral image, calculating the standard deviation image, and performing a line of additional noise removal and thresholding (Fig. 1D). Images were aligned using the image of the vascular mask with NIH Image plug-in TurboReg. The vascular mask also served to isolate the vascular field for analysis by PIV.

Microvascular Image Velocity Imaging (Micro-PIV). Micro-PIV (min-PIV) was performed on B1-B4 image stacks using the NASA PIV/PIC software program to measure local velocities at each pixel in the field of view. Micro-PIV records the displacement of red blood cells by calculating the cross-correlation coefficient between adjacent images. Micro-PIV is an optical method that tracks movement and deformation of particles and blood cells (red, white, and platelets, respectively). Background velocities (i.e., velocities in tissue outside of the blood vessels) within an image stack were set to zero using the microvessel mask (Fig. 2B). PIV/PIC employs advanced techniques and complex image processing operations following background subtraction, and uses fuzzy logic for validating the identification of correlation peaks and particle pairing in particle-tracking operations.

Analysis of Blood Flow Velocity and Volumetric Flowrate. To resolve the flow patterns resulting from periodic vessel cycles due to cyclic pumping of the heart, the velocity field generated by PIV/PIC for each image within a 15-fitting stack of B1-B4 was averaged over seven time steps (i.e., seven images before, and three images after the analysis image). The velocity field of blood flow in an image was converted by the scientific visualization software imageJ to a correlated map of velocity vectors. Average blood flow velocity further confirmed by analyzing the whole field of view and for the entire vascular network. The volumetric flowrate was calculated at each cross-section of a vessel by numerically integrating the velocity profile.

RESULTS
Results for a B1-B4 image set from a representative experiment with one specimen are illustrated in Figs. 3.4 Cross-sectional velocity profiles in the postcapillary collecting veins were approximately parabolic, indicating laminar blood flow. According to mean values of the cross-sectional radius (red, green, and blue), the volumetric flowrate of 1.30 μl/min at maximum velocity across the largest in the B1-B4 diameter (169 μm) decreased to 1.7 μl/min at minimum (27 μm) velocity. The maximum volumetric flowrate in the largest vessel in the B1-B4 (diameter = 74 μm) was 6.0 ± 2.0 μl/min. Overall, the maximum flow velocity at the center of the vessels for B1-B4 decreased approximately 20%, from a maximum of 2000 μm/sec for the largest vessel in B1-B4 to 100-150 μm/sec in smaller capillaries (e.g., see B4). Absolute differences in the spatial average of velocity over time declined considerably from B1 to B4. Pulsatile flow cycles in B1–B4 were approximately equal (0.6 sec).

DISCUSSION
By intravital micro-PIV, velocity flow fields were investigated for the first four branching orders of the microvascular network in the zebrafish embryo at E5. Blood flow was measured by tracking RBCs as the moving particles. Parabolic velocity profiles in the larger veins indicate that blood flow was laminar. Measurements of individual vessels without volume, blood velocity and volumetric flowrate decreased with increasing vessel size (Fig. 3). We see qualitatively consistent with the Hagen–Poiseuille law. In future calculations, we will investigate the mathematical relationship between vessel diameter, velocity, and volumetric flowrate in our experimental model. Spatial averaging of the velocity flow field (Fig. 4), absolute differences in maximum and minimum averaged velocities for periodic flow cycles also decreased with decreasing branching order (Fig. 4). We conclude our study by segmenting circuital vessels and vessels in the velocity flow fields of B1-B4 using the OIC Software VEGAS.15 Segmentation classification of the vessels into vascular branch orders will support further morphological analysis of blood flow dynamics as determined by vessel parameters that include vessel diameter, branch point density, and tortuosity.

Figure 1. Microvascular branching and blood imaging. (A) Fetalisized eggs were cultured on coverts, oval-Petri dishes (Column: Cox: jackson) were cultured between 37.5°C and 32°C. All embryonic day 5 (E5), a field of view was selected from the yolk sacs, velocitography (Fig. 1A) for the imaging of four successive vessel branching orders (E5-B1-B4) that included successive vascular branching generations and capillary venules (Fig. 1B). The Petri dish was placed on a microscope stage, warmed to 37°C and then under an infrared light trap. A digital image stack (Fig. 1C) was acquired by 256 images (image size 1024 x 1024 pixels at 125 frames per second) at vessel spacing was acquired in propap (250 levels) at a total magnification of 14× (resolution = 0.07 μm/pixel) with an Olympus BXZ2 stereo microscope and a high-speed, high-magnification Photonic Image Company MPPS digital camera and Photonic FastCompare PIV (FC-PIV) software, illuminated by a 100W microscope lamp with a 9U filter. A set of four-image stacks, B1-B4, was acquired from each of the embryos. Each representative image set was reported here. Images were processed by subtracting each image from itself to provide a clear image obtained by analyzing the image stack with block and vessel tracking software in PIV analysis. Noise was reduced by adjusting the adjuster, a single pixel tracking pixel only displaying the vessel field was selected by scanning the aligned images as an integral image, calculating the standard deviation image, and performing a line of additional noise removal and thresholding (Fig. 1D). Images were aligned using the image of the vascular mask with NIH Image plug-in TurboReg. The vascular mask also served to isolate the vascular field for analysis by PIV.

Figure 2. Imaging and Tracking of a Blood Flow. (A) A paracrine image from the B2 image stack illustrates vascular morphology and blood flow (B). The paracrine vessel mask of the B2 image stack was used (C) to define the image stack and (D) to evaluate the analysis of blood flow velocity by PIV.

Figure 3. Analysis of Velocity Flow Fields for Successive Vessel Branching Generations. Velocity flow fields measured by micro-PIV and averaged for volumes of peak flow are illustrated for B1-B4 (A-D), where the radiance of the velocity vector contours are proportional to color coding (red, blue, yellow), respectively, the image shows three-dimensional and two-dimensional views of the velocity field, as generated by cross-sectional reds (red, green, and blue circles), and are shifted to the upper right corner of the figure panels. The white arrow indicates the direction of vessel flow, and the white lines show sub-vessel scale for the B4 vessels. Average velocities decreased considerably with decreasing vessel diameter. Velocity profiles are highly parabolic, redgling that blood flow within the vessels was laminar. Cyclic pulsatility of the various blood flow circulation is displayed in graph of average velocity versus time (gast card).