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Multi-image particle tracking velocimetry of the microcirculation using fluorescent nanoparticles

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Abstract

Particle tracking velocimetry provides a Lagrangian description of flow properties in the microcirculation. To determine the utility of fluorescent nanoparticles to provide Lagrangian coordinates, we tracked these particles both in vitro and in vivo. The particles had a neutral charge and fluorescence intensity greater than 1000 times the PKH26-labeled red blood cells. At image acquisition rates of 60 frames per second, particles were tracked at velocities up to 4000 μm/s. Morphometric changes reflecting streaking artifact were significant at velocities of 4000 μm/s ($P < 0.05$), but not at lower velocities ($P > 0.05$). Intravital microscopy monitoring after intravenous injection of the particles demonstrated a circulation half-life that was inversely related to particle size: 500 nm nanoparticles demonstrated a smaller change in plasma concentration than larger particles. Regardless of the size of the particles, more than 50% of the recovered fluorescence was located in the liver. These results suggest that fluorescent nanoparticles provide a convenient and practical Lagrangian description of flow velocity in the microcirculation.

Keywords: Microcirculation; Nanoparticles; Fluorescence microscopy; Velocimetry

Introduction

Studies of blood flow regulation have focused on the accurate description of the velocity field because other flow property calculations follow directly from these measurements. To obtain a detailed description of the velocity field in the microcirculation, conventional velocimetry techniques consider the field to be composed of a very large number of particles (Raffel et al., 2000; Westerweel, 1997). The spatial displacement of these particles in two separate images provides a measurement of the instantaneous in-plane velocity vector field (Adrian, 1984, 1991). These instantaneous measurements provide a "snapshot" description of the velocity field. Commonly referred to as Eulerian methods, these descriptions provide a highly resolved and computationally manageable velocity profile at a given point in space and moment in time.

In contrast to Eulerian methods, Lagrangian methods track the movement of individual particles. Particle tracking as a function of time provides a limited description of the velocity field at a particular point in space but more information regarding the fate of individual particles. Thus, Lagrangian descriptions may be especially useful in studies of leukocyte behavior in the microcirculation (Secomb et al., 2003; Su et al., 2003; West et al., 2001). Leukocyte trajectories that involve margination, mural interactions and prolonged residence times are best characterized using the computation of Lagrangian coordinates.

In this report, we investigated the use of submicron fluorescent spheres, here termed nanoparticles, for microcirculatory particle tracking. The motion of the nanoparticles was investigated both in vitro and in vivo. Our findings suggest that

Abbreviations: CCD, charge coupled device; RBC, red blood cells.

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intravital videomicroscopy and nanoparticles can provide accurate Lagrangian flow description of the microcirculation observed in vivo.

Methods

Particles and red blood cells

The particles were developed by Molecular Probes (Invitrogen, Eugene, OR) for intravascular particle tracking. These particles were of similar composition to those reported previously (Bernard et al., 1996) but manufactured with superior fluorescent characteristics and smaller size. Although many different fluorescent colors were developed, the green (e.g., 490 nm; em 520 nm) and orange (e.g., 545 nm; em 570 nm) fluorescent nanoparticles were used for most experiments. Sheep red blood cells were obtained in a heparinized syringe and separated from white cells by density gradient centrifugation. The red cells were fluorescently labeled using the procedure included in the commercially available PKH26 Red Fluorescent Cell Linker kit (e.g., 551 nm; em 567 nm) (Sigma, St. Louis, MO). Based on empirical preliminary findings, the number of injected nanoparticles was normalized to the volume of red blood in 1 ml; namely, 452 μm^3. The number of injected nanoparticles was based on an equivalent total spherical volume: 6.912×10^9 500 nm particles, 1.08×10^8 2 μm particles and 1.3×10^7 4 μm particles.

Electronic particle and cell volume

Electronic particle volume analysis was performed using a Coulter Z2 Particle Analyzer (Beckman Coulter, Miami, FL). The Coulter Z2, based on the Coulter principle (Ben-Sasson et al., 1974; Brecher et al., 1956), measured changes in electrical resistance produced by the nonconductive particles and cells suspended in a standard electrolyte solution (Isoton II; Beckman Coulter). A 50-μm aperture was used with constant voltage settings. Particle minimum and maximum diameter settings were modified for the analysis. The particle size and number distributions were recorded over 256 channels and exported to Microsoft Excel (Redmond, WA) for statistical analysis.

Flow cytometry

The nanoparticles and PKH26-labeled red blood cells were analyzed on an Epics XL (Beckman Coulter, Miami, FL) using gain settings calibrated to 4 peak Rainbow calibration particles (Spherotech, Libertyville, IL). The data were processed using WinList 5.0 (Verity, Topsham, ME).

In vitro flow chamber

The design of the flow chamber has been previously described (Li et al., 1996, 2001). Briefly, the chamber was machined from high-grade acrylic to minimize optical aberration and facilitate microscopy. Design features included 0.5-mm holes in both inlet and outlet manifolds to rapidly stabilize laminar flow and permit the use of standard microscope slides. At each end of the flow deck, rounded fluid capacitors dampened eddy currents at the higher flows. The flow chamber was perfused with an NE-1000 withdrawal syringe pump (New Era, Farmingdale, NY). In most experiments, the perfusate was normal saline containing either particles or red blood cells.

Optical system

The optical systems were Nikon Eclipse TE2000 inverted epifluorescence microscopes using Nikon CFI Plan Fluor ELWD 10×, 20× and 40× objectives. The intravital microscopy system used a Nikon Fluor WD 20× objective. An X-Cite (Exfo, Vanier, Canada) 120-W metal halide light source and a liquid light guide was used to illuminate the tissue samples. Excitation and emission filters (Chroma, Rockingham, VT) in separate LEP motorized filter wheels were controlled by a MAC5000 controller (Ludl, Hawthorne, NY) and MetaMorph software 6.26 (Molecular Devices, Brandywine, PA).

Electron multiplier CCD (EMCCD) camera

The flow chamber and intravital videomicroscopy 14-bit fluorescent images were digitally recorded with a EMCCD camera (C9100-02, Hamamatsu, Japan). The C9100-02 has a hermetic vacuum-sealed air-cooled head and on-chip electron gain multiplication (2000×). Images with 1000 × 1000 pixel resolution were routinely obtained at 30 fps; frame rates exceeding 60 fps were routinely obtained with 2 × 2 binning or subarray acquisition. The images were recorded in image stacks comprising 30 s to 10 min video sequences.

Calculation of diffraction-limited resolution

Using matching apertures of the objective and condenser, the radius of the first order Airy diffraction ring was calculated using the formula

$$r = 1.22\lambda/2\mathrm{NA},$$

where λ is the wavelength and NA is the numerical aperture of the objective. The minimum resolved distance between Airy patterns (Rayleigh criterion) was calculated as

$$r = 0.61\lambda/\mathrm{NA}$$

and was used to determine maximum concentration of the particles in flow chamber experiments. The concentration of red cells and particles was, at maximum, 10-fold less than the concentration defined by the Rayleigh criterion.

Quantitative morphometry

Out-of-focus blurring and camera-dependent streaking was assessed using quantitative image analysis (MetaMorph; Molecular Devices, Downingtown, PA). The out-of-focus effects were assessed by optical dispersion. The *optical dispersion* was a measure of the total fluorescence intensity around the centroid of the particle. The camera-dependent streaking was assessed by the elliptical form factor. The *elliptical form factor* was calculated as the ratio of a particle's breadth to its length.

Mice

Male Balb/c mice (Jackson Laboratory, Bar Harbor, ME), 25–33 g, were used in all experiments. The care of the animals was consistent with guidelines of the American Association for Accreditation of Laboratory Animal Care (Bethesda, MD).

Multi-frame particle tracking

Particle tracking was performed on digitally recorded and distance calibrated multi-image "stacks." The image stacks produced a sequential time history of velocity and direction as the acquired images were time stamped based on the 100 mHz system bus clock of the Xeon processor (Intel, Santa Clara, CA). The movement of individual particles was tracked using the MetaMorph (Molecular Devices) object tracking applications. The intensity centroids of the particles were identified and their displacements tracked through planes in the source image stack. For displacement reference, the algorithm used the location of the particle at its first position in the track. Each particle was imaged as a high contrast fluorescent disk and its position was determined with subpixel accuracy. The image of the particle was tracked using a cross-correlation centroid-finding algorithm to determine the best match of the cell position in successive images. The resulting measures included the x and y coordinates, velocity, mean displacement, mean vector length, mean angle (the angle of the mean vector of the object) and the angular deviation.

Ear microscopy

The ear intravital microscopy was performed by using a custom-machined titanium stage (Miniature Tool and Die, Charlton, MA). The tissue contact area consisted of a 2-mm vacuum gallery that provided tissue apposition to the stage surface without compression of the tissue and with minimal circulatory disturbances.