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Analysis of the Cell-Free Layer in a Circular Microchannels: Trajectories of Labeled Red Blood Cells

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Abstract

In this experimental work, wemeasure the trajectories of the cell-free layer (CFL) by tracking labeled red blood cells (RBCs) flowing around the boundary of the RBCs core.The circular glass microchannels studied are 100μm in diameter. The images are captured using a confocal system and are post-processed using Image J and MATLAB. The results suggest that the trajectories followa polynomial function.

1. Introduction

The cell-free layer (CFL) is well known physiological phenomenon that happens at both in vivo in vitro blood flowing in microcirculation [1-4]. This phenomenon is due the red blood cells (RBCs) tendency to undergo axial migration due to the high shear stress around wall that forces the RBCs to move towards the center of the channel. Although there have been several studies on the measurement of CFL thickness, according to our knowledge there have been very few studies on the determination of CFL trajectory. The main purpose of the present work is to measure the measure several trajectories of the CFL by tracking labeled RBCs in 100 μm glass cappillaries. This experimental study was performed using a confocal microscopy system together with image analysis techniques.

2. Materials and Methods

2.1. Working fluids and microchannel geometry

The working fluid used in this study was Dextran 40 (Dx-40; Otsuka Medicine) containing 12± 2% (12Hct) of human RBCs. The Hcts corresponded to the feed reservoir Hct and were measured using a hematocrit centrifuge (Kubota 3220)immediately before each experiment. The RBCs were labeled with a lipophilic carbocyanime derivative, chloromethylbenzamido (CM-Dil, C-7000, Molecular Probes). A detailed description about the procedure for labeling the human RBCs can be found elsewhere [3].

The microchannels tested in this study were 100-μm circular borosilicate glass microchannel fabricated by Vitrocom (Mountain Lakes). The microchannel was mounted on a slide glass was immersed in glycerol to minimize the refraction from the walls.

2.2. Experimental set-up

The confocal micro-PTV system used in this study consists of an inverted microscope (IX71; Olympus) combined with a confocal scanning unit (CSU22; Yokogawa), a diode-pumped solid-state (DPSS) laser (Laser Quantum) with an excitation wavelength of 532 nm and a high-speed camera (Phantom v7.1; Vision Research). The microchannelswere placed on the stage of the inverted microscope and by using a syringe pump (KD Scientific) a pressure-driven flow was kept constant (Re ~ 0.008). Additionally, by using a thermo plate controller (Tokai Hit) the temperature was set to 37°C±1. More detailed information about this system can be found elsewhere [3, 5, 6].

2.3. Tracking RBC trajectory

The laser beam was illuminated from below the microscope stage through a dry40× objective lens with a numerical aperture (NA) equal to 0.9. The confocal images were captured in middle of the capillary with a resolution of 640×480 pixel at a rate of 100 frames/s with an exposure time of 9.4 ms.A manual tracking plugin (MTrackJ) [7]of an image analysis software (Image J, NIH) [8] was used to track the label RBCs. By using MTrackJ plugin, the bright centroid of the selected RBC was used through successive images. After obtaining series of x and y positions, data were exported for MATLABand the *cftool* package was used to calculate the best function that approximates the numerical results. Fig. 1 shows the trajectories of a labeled RBC flowing around the edge of the RBCs core.

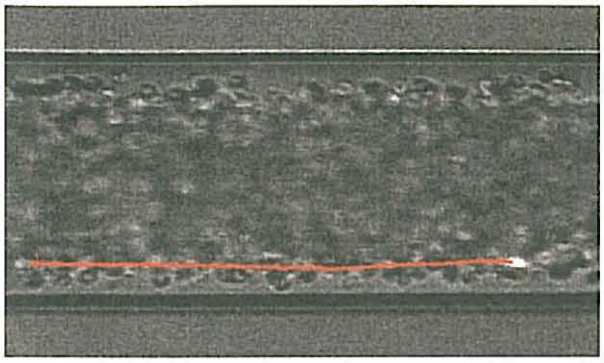

Fig. 1.Trajectory of a labeled RBC flowing around the boundary region between CFL and RBCs core.

3. Results and Discussion

This section presentsnumerical results concerning to the approximation of the data that was obtained using the technique described in the previous section.

We used the *cftool* package of MatLab softwarewhich is identified as the best function that approximates the data using the least squares technique. In the following table it is presented the error of least squares technique of the seven best approximations.

Table 1. Error of the seven best functions obtained with *cftool*.

Function	Error (um)			
Poly5	**18,6327**	**6,8002**	**22,9216**	**18,0606**
Fourier1	**21,6219**	11,0451	**28,1591**	**20,6800**
Poly4	**22,9811**	**7,7465**	**26,8842**	**20,2669**
Poly3	**23,7068**	**8,2364**	**33,9576**	**24,1843**
Exp2	23,7505	**8,1408**	34,0389	24,6697
Poly2	23,7738	12,2127	33,9702	25,7616
Power2	24,3397	24,6196	43,3759	51,3068

Considering the data of one cell, Fig. 3 shows the five best functions obtained with least squares method.

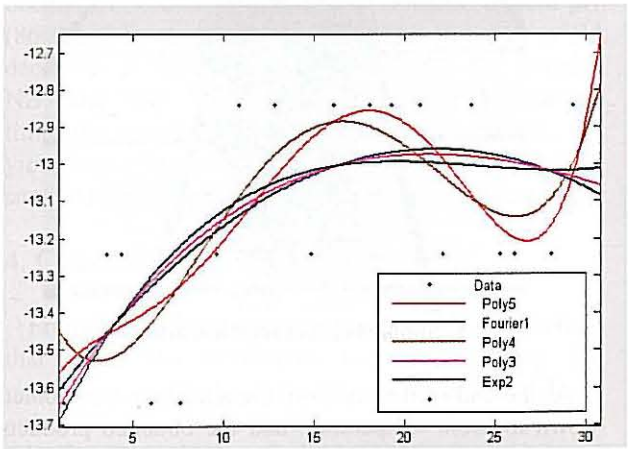

Fig. 3. Graph of the five best functions that define the trajectory of one cell.

Observing the Table 1 and Fig. 3 we can conclude that the best function that approximate the data is based on polynomial of degree five.

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