MEASURING ERRATIC MOTIONS OF LIGAND-COATED MICROSPHERES IN AN IMPOSED ENERGY FIELD

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ABSTRACT

Suspensions with fluorescent PSGL-1 (P-selectin glycoprotein ligand-1) microspheres and with or without red blood cells (RBC) were perfused through a parallel plate flow chamber at physiologically relevant wall shear rates to quantify more explicitly the effects associated with cell-induced erratic energy transfers. Using a moving chamber on a fluorescence microscope, microspheres were observed to roll along the coated upper surface of the chamber and to follow the relationship with respect to a traveling reference point:

$$l^2 \sim (K)(-t/a\text{-}1+e^{t/a}) \quad (1)$$

where l^2 is the square of the distance to the reference point, t is the average first passage time to +/- l, K is a dimensional constant, which is assumed to relate to the mass and friction forces in the system, and a is a scaling constant. The RBCs affected the rolling behavior of the microspheres in a manner consistent with prior reported work. The time scale for the RBC data is much smaller than the time scale for the microspheres alone. It quantifies the readily visible finding: the presence of RBCs in the suspension caused the microspheres to roll faster and larger fractions of them to exit the images on the downstream end.

INTRODUCTION

Rolling of leukocytes along the endothelium is considered a classic step in any inflammatory response cascade. Most *in vitro* studies of leukocyte rolling have ignored the continuing energy exchanges of a traceable body associated with its many interactions among individual bodies and material in the bulk solution and with the reactive boundary. Continuing energy exchanges in these situations, which involve much mutual, directed motion, mandatorily includes erratic events in the particle paths. The "unevenness" of the axial progress occurs with regard to a local condition, which presumably can be traced by use of a moving reference point and thus included in image analysis related to experimental measurements. Recognizing this inherent erratic nature of all particle paths permits a focus on defining multiple zeroes along individual paths. Multiple zeroes are easily determined for our experiments by the use of a moving reference frame. They occur as a part of changes of position over various lengths, l, in the axial direction of the imposed energy field associated with the flow. When the speed of a rolling microsphere matches the speed of the stage, the microsphere appears stationary (a zero). These multiple zeroes give rise to the pause time distribution discussed by Zhu [1]. The fitting method provides a way to assign two constants to the observations.

The ligand-receptor pair PSGL-1 – P-selectin was chosen for this project because PSGL-1 is constitutively expressed in an epitope that is common to all leukocytes [2]. PSGL-1 has also been shown to bind all three known selectins: E-, L-, and P-selectin. Binding to E- and P-selectin is known to promote rolling of leukocytes along the endothelium, and binding to L-selectin may help recruit leukocytes to an inflamed area.

THEORY

The motions of the rolling microspheres are analyzed in the context of First Passage Measurements. These techniques are based in the idea of a random walk model. Random walks allow continuous phenomena to be modeled as discrete phenomena, and these models are classified according to their treatment of certain variables as fixed or random and the probabilities associated with the variable of interest. The algebraic form used here, a relationship between averages of increments of relative displacement and of times for the displacement, is shared by models of persistent random walk and momentum diffusion (Ornstein-Uhlenbeck processes). Here, a negative sign for the time increments must be used to obtain strong statistical fits. The data thus are summarized by an exponential growth, a description that fits an unstable distribution. This "explosive" mathematical form is a natural mode when the goal of the model is describing a rolling process that precedes arrest and hopes to move to transmigration.

MATERIALS AND METHODS

Brief descriptions of each type of experiment are given below. PSGL-1 microspheres were prepared with a detailed procedure

developed by Goetz et al. [3,4]. Images were collected in flow experiments on a moving stage of a videomicroscope using methods developed by Leggas [5].

Microspheres Only

Five mL of a 0.003% (by volume) suspension of 10-μm-diameter fluorescent microspheres (Molecular Probes, Eugene, OR) were drawn from a reservoir at atmospheric pressure through an "inverted" parallel plate flow chamber at a wall shear rate of > 100 s^{-1} (chamber dimensions: 17.145 cm L × .3175 cm W × 0.01778 cm H). The upper surface of the flow chamber comprised a glass coverslip coated with 250 μL of 20 μg/mL recombinant human P-selectin (R & D Systems, Minneapolis, MN) [4]. The stage speed was set at 1 mm/sec so as to bracket the rolling speeds of the microspheres. Images of the flow were videotaped under 4× magnification (objective) and analyzed off-line with Inovision's "ISEE" software (Raleigh, NC). The intensities of the rolling microspheres' images were used to generate the x- and y-positions of the microspheres frame by frame. The collection of files for each experiment was processed using MatLab (The MathWorks, Inc., Natick, MA) programs written by Mr. Baoshun Ma. Separate programs returned an average time for all of the microspheres to cross each fence of predetermined distance and the fit coefficients for the sets of time and distance data.

Microspheres plus Red Blood Cells

One mL of guinea pig RBCs was added to the microsphere suspension described above to prepare a 20% (by volume) solution of 0RBCs. The remainder of the experiment was performed as for the suspension that consisted of only microspheres.

RESULTS AND DISCUSSION

Data obtained in early trials are shown in Figure 1. This figure shows the average first passage times from both types of experiments for 15 selected fence distances. Solid lines through the different sets of data points, which are sometimes hidden by the density of points, show the fits for the proposed two-fitting constant model. Both of the fits shown for the microspheres-only data are to the proposed mathematical form. The second fit shown considers only the first ten fence distances, however, and serves to provide insight into the nature of the distribution of the data around the analytical design. Eight microspheres were analyzed from the microspheres-only suspension, and six microspheres were analyzed from the suspension with RBCs with this experimental design. Data sets for both microspheres-only and microspheres plus RBCs suspensions show good fits to the proposed mathematical form. The average first passage times scale in the same manner in both the absence and presence of RBCs. The presence of RBCs augmented the interactions with the reactive boundary in a consistent manner as well. The microspheres rolled faster in the presence of RBCs and exited the field of view preferentially downstream.

These fits of early data suggest the feasibility of experimental techniques to collect and analyze images of rolling microspheres and cast the results in a form of the proposed two-fitting-constant model. The addition of RBCs to the microspheres suspension enhanced the interactions of the microspheres with the wall in a manner consistent with the bulk behavior for cellular paths and energy loss, generally cited as Fåhraeus and Fåhraeus-Lindqvist effects. New methods of data collection and analysis are more easily implemented and "look" better in early tapes; they also bring parameters such as apparent protein density for the coverslip coating toward better agreement with *in vivo* conditions. Work is underway to determine the influence of the microsphere coating density and the selectin coating density on the fit parameters. Preliminary data indicate that the *in vitro* measurement techniques discussed here work even better as the selectin coating density is adjusted to match published values for *in vivo* conditions. Such new data will be analyzed and available for the meeting.

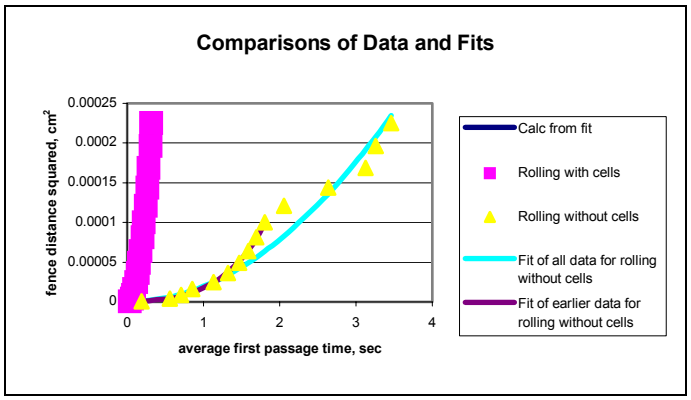

Figure 1. Rolling microsphere data and fits for all of the experiments.

CONCLUSIONS

The experimental method permits the identification and analysis of labeled rolling microspheres in suspensions with physiologically pertinent hematocrits. The current analysis shows that the RBCs increase the rolling velocity of the microspheres and provides a means to quantify the statistical situation regarding imposed, net energy transfer. Rolling microspheres in both the presence and absence of RBCs show good fits with the proposed mathematical form.

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