# DETERMINING SPATIAL DISTRIBUTION OF FLOWING CELLS ADHERENT TO SURFACE: THE BINARY ADHESION CASE

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## INTRODUCTION

The adhesion of flowing cells to the vascular surface in localized sites occurs in many physiological and pathological conditions, such as inflammatory reaction, thrombus formation, and tumor metastasis. The spatial and temporal distributions of such adhesion depend on the expression and regulation of adhesive receptors and ligands that mediate the specific interactions between the flowing cells and the endothelial cells lining the vessel wall, the reaction kinetics of the interacting molecules, and the hemodynamics of blood flow by which the cells are transported. As a first step toward developing a framework for exploring the action and interplay of these factors, we describe here a minimal model and its experimental validation.

## THEORY

The minimal model only treats binary adhesion or irreversible association, i.e., the cell will be arrested once it forms the first bond with the surface and it will not dissociate. As such, it contains only on-rate for receptor/ligand association because the off-rate is zero. Let $N_b$ be the number density (in $\mu m^{-2}$) of adherent cells and $\psi$ be the number density of flowing cells sufficiently close to the surface such that interactions become possible. They are related by the kinetic equation

$$\frac{\partial N_b}{\partial t} = k_{ad}\psi \tag{1}$$

where $k_{ad}$ is a rate constant for cell association, or a cellular on-rate (in $s^{-1}$). The dependence of $N_b$ on position $x$ and $y$ on the surface and time $t$ provides a complete description of the spatial and temporal distribution of adhesion. $\psi$ is defined as the integral of the cell concentration (number of cells per unit volume) over the thickness of a very thin "boundary layer" of flowing fluids immediately above the chamber floor. The cells within the boundary layer are considered capable of tethering (but have not yet tethered) to the surface. Further above the boundary layer the cells are assumed to be too far away from the surface to bind. $\psi$ changes as the cells are transported downstream because some are removed from the boundary layer by binding (efflux at the bottom) and others are added by sedimentation (influx at the top). Such changes are described by the following equation of the conservation of cells:

$$\frac{\partial \psi}{\partial t} + \frac{\partial (u\psi)}{\partial x} + \frac{\partial (v\psi)}{\partial y} + k_{ad}\psi = w_c C \tag{2}$$

where $u$ and $v$ are fluid velocity components along the $x$ and $y$ directions (averaged over the boundary layer thickness), respectively. $w_c$ is the $z$ component of cell velocity in the $z$ direction (vertically downward toward the surface). $C(x,y)$ is the concentration of the following cells on the upper surface of the boundary layer.

The cellular on-rate $k_{ad}$ is an aggregate parameter that lumps the forward-rate of molecular interaction, $k_f$, with the densities of the receptors and ligands, $m_r$ and $m_l$, on the respective cell membrane and vessel surface, the collision frequency $f_c$, the contact time, $t_c$, and contact area, $A_c$.

$$k_{ad} = 1 - \exp(-m_r m_l k_f A_c t_c f_c) \tag{3}$$

It can also be directly measured according to

$$k_{ad} = (n/T)/(mu T/L) \tag{4}$$

where $n$ is the number of adherent cells observed in an area during time $T$, $m$ is the number of cells appeared in the same area at the instant when a cell is observed to tether to the surface, $u$ is the velocity of the following cells and $L$ is the length of the observation area in the direction of flow. Equations 1-4 are the basis for predicting the adhesion pattern $N_b$.

## EXPERIMENT

Fcγ receptor-mediated neutrophil adhesion to plastic surface coated with IgG in a parallel-plate flow chamber was used as an experimental model to test our theory because this adhesion is observed to be binary [1]. This model also is relevant to several chronic inflammatory disorders such as rheumatoid arthritis and systemic lupus erythematosus where neutrophils adhere to vascular surfaces coated by immune complexes [2,3].

Neutrophils were isolated from freshly collected blood from a healthy donor and their surface expression of Fcγ receptors ($m_r$) were determined by radioimmunoassay (RIA). The IgG densities ($m_l$) on the

chamber floor were also determined by RIA. The flow chamber has a channel width of 1 cm, and length of 4 cm, and a thickness of 200 µm. At each flow rate, the total number of cells passed through a non-adherent flow chamber was determined by directly counting the cells discharged. The cellular on-rate $k_{ad}$ was measured as a function of the IgG density and cell velocity according to Eq. 4. The adhesion pattern $N_b$ was determined by enumerating adherent cells in 8 fields of view at any given position along the flow direction ($x$ axis). Data were taken in 0.5 cm increments from the inlet ($x = 0$) to exit ($x = 4$ cm).

## RESULTS

Under a wide range of conditions the model solution is capable of globally fitting the measured adhesion pattern for a range of wall shear stresses and IgG site densities, as exemplified in Fig. 1 for $m_l = 28{,}000$ IgG/µm$^2$. The fitting returns $k_{ad}$ as a function of the wall shear stress and IgG site density, as shown in Fig. 2, which compares reasonably well with direct measurements.

Despite the fact that the same number of cells (0.5 million) suspended in the same volume (1 ml) were sent to the flow chamber in all experiments, it was found that only a fraction of cells made it through the flow chamber, which increases with wall shear stresses, as shown in Fig. 3.

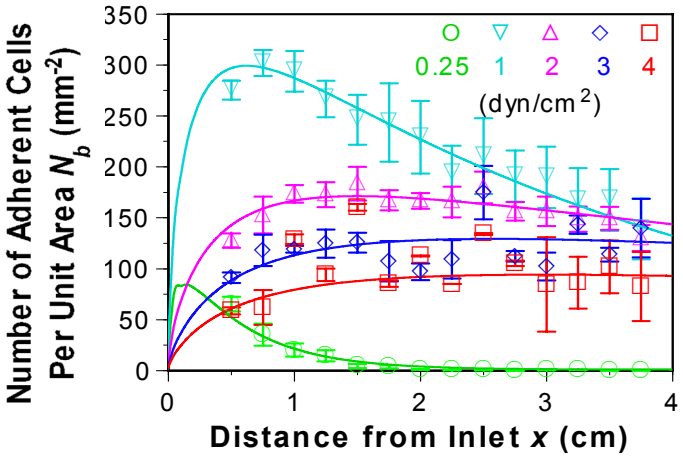


**Figure 1 Comparison between measured (points) and fitted (curves) of spatial distribution of adherent cells along the direction. Data are presented as mean ± SEM of 8 fields of view.**

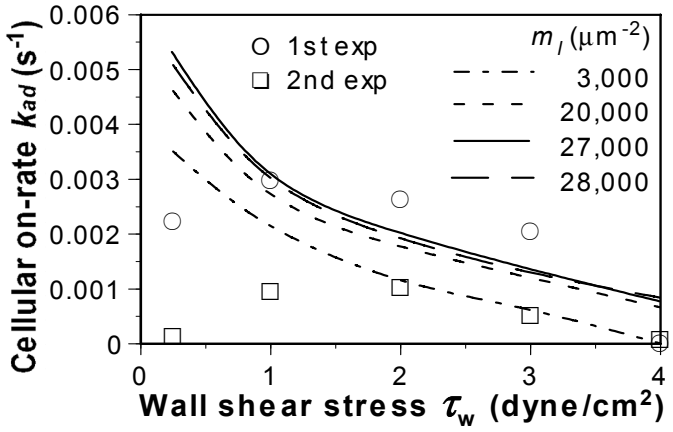


**Figure 2 Comparison between directly measured (points) and predicted (curves) cellular on-rate $k_{ad}$ as a function of wall shear stress and IgG density. Experiments were done at $m_l = 20{,}000$ µm$^{-2}$**

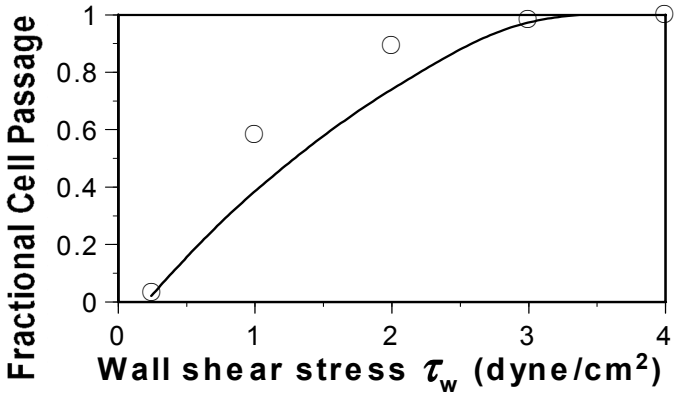


**Figure 3 Comparison between measured (points) and predicted (curve) fractional cell passage through the flow chamber**

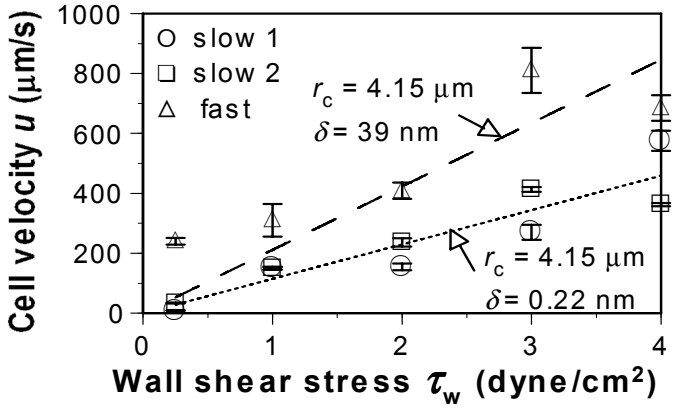


**Figure 4 Measured and fitted cell velocity near the chamber floor as a function of wall shear stress. $r_c$ = cell radius. $\delta$ = gap distance.**

The measured cell velocity was shown in Fig. 4. Two populations were noted, one with slow and the other with fast velocities. Fitting the Goldman theory [4] to these return two gap distances between the surface and the cell bottom, which defines the thickness of the boundary layer where cells are sufficiently close to the surface for binding to be possible.

## ACKNOWLEDGMENTS

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