Response to the Letter by King and Hammer

In their letter, King and Hammer present results from their previously published article on hydrodynamic recruitment (King and Hammer, 2001), and comment on its relationship with our recently published manuscript (Zhang and Neelamegham, 2002). The objective of our response is to clarify the findings of our work in the context of this letter. Two specific points are addressed; the first pertains to our calculations of flow disturbance due to an adherent cell, and the second to our experimental data presented in Supplemental Data (http://www.eng.buffalo.edu/~neel/pplate.html).

With regard to the first point, we agree that "hydrodynamic recruitment" (as defined in King and Hammer, 2001) of rolling leukocytes cannot be accounted for by purely considering changes in the local shear rate due to a bound cell. As seen in Appendix 1 of our article (Zhang and Neelamegham, 2002; corrections to appendix derivation are posted with supplemental material at the author's website), our calculations describe the disturbance to the local flow due to a bound cell, while neglecting the second cell in its vicinity. This derivation also clearly states that we have neglected hydrodynamic wall effects, and that accounting for this feature would further reduce the disturbance caused by the bound cell. We chose to neglect this feature since we were interested in presenting a simple, albeit approximate, *analytical* solution of flow that is convenient for an average experimenter to use. Further, we state in the article that ~2.5 cell diameter represents the outer bounds of the region where the local shear is altered by at least 5%. The letter from King and Hammer suggests that our calculations are approximate, without acknowledging that our model assumptions are clearly stated in the original manuscript. Taking the conclusions of our work on flow disturbance with the work of King and Hammer (King and Hammer, 2001), we may conclude that hydrodynamic recruitment as defined by these authors cannot be accounted for based on flow-disturbance calculations alone.

With regard to the second point, we do not believe that the results presented in Supplemental Data directly support, or weaken, the proposition that hydrodynamic recruitment is a significant phenomenon. As King and Hammer point out, and we agree, our experiments in Supplemental Data do not probe hydrodynamic recruitment. This was not our intention when we performed the experiments, nor do we make any such statement in our article. In our opinion, probing "hydrodynamic recruitment" is a difficult task in cellular assays. This would require the development of new statistical tests to distinguish 1) between the roles of hydrodynamic recruitment and L-selectin bond formation on leukocyte secondary capture, and 2) between hydrodynamic recruitment and primary cell capture. Experiments would have to be performed where L-selectin (and perhaps even the β_2-integrins at low shear) is blocked, with Fab fragments of blocking antibodies or other inhibition strategies, to distinguish between the rates of pure hydrodynamic recruitment and primary capture. Additional runs in the absence of the above blocking strategies would be required to establish the degree to which hydrodynamic recruitment acts in synergy with L-selectin bond formation to mediate secondary capture. Interpretation of such experiments may be complicated since specific strategies to block hydrodynamic recruitment without affecting adhesion molecule function are not currently established to the best of our knowledge.

Since we do not implement the above strategies in Supplemental Data, we respectfully disagree with the proposition of King and Hammer that "the Supplemental Data of Zhang and Neelamegham do contain an observation that reveals that hydrodynamic recruitment is indeed an important mechanism in in vitro flow assays." Our approach of examining secondary capture involves estimation of a parameter called cell-cell capture probability (θ_{cc}) from experimental data, which we define as the fraction of collisions between cells in the free-stream and previously adherent cells that result in capture. This is a lumped parameter that incorporates all the features contributing to secondary capture, including receptor-mediated leukocyte-leukocyte tethering and the effects of fluid flow.

We thank King and Hammer for their comments and for this stimulating discussion.

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