# *Interstitial Flow and Effects on Tumor Cell Migration*

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# INTERSTITIAL FLOW AND EFFECTS ON TUMOR CELL MIGRATION

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## INTRODUCTION

Interstitial flow is the convective transport of fluid through tissue extracellular matrix. This creeping fluid flow has been shown to be important in regulating the development, function, and pathology of tissues. Furthermore, interstitial flow has been shown to affect the morphology and migration of cells such as fibroblasts, cancer cells, endothelial cells, and mesenchymal stem cells (1). Chary and Jain used fluorescence recovery after photobleaching to directly observe fluid flow in the tissue interstitium and determined typical flow velocities are on the order of 0.1-2.0μm/s (2). Interstitial flow is particularly important in driving transport in tumor tissues, as neoplastic tissue is often characterized by increased interstitial pressure (3).

Understanding the details of the cellular response to interstitial flow is complicated by the myriad of cell types and intricate flow patterns characteristic of *in vivo* tissue. Consequently, *in vitro* systems have been developed to isolate the effects of pressure gradients and flow on cell physiology (4-6). Shields et al. developed a modified Boyden chamber assay and demonstrated that interstitial flow increases the metastatic potential of tumor cells. The authors demonstrated the increased metastatic potential was activated through binding of self-secreted ligand to the CCR7 receptor (4). This autocrine signaling mechanism, called autologous chemotaxis, arises in a flow field where convection distributes autocrine factors and leads to a transcellular concentration gradient, which in turn, provides a chemotactic signal (7). However, details of the autologous chemotaxis response are not well understood because of limitations in the Boyden chamber assay.

We developed a microfluidic cell culture system in which we can apply stable pressure gradients and fluid flow, and in which we can observe transient responses of breast cancer cells seeded in a 3D collagen type I scaffold. We employed this system to examine cell migration in the presence of interstitial flow to address the hypothesis that interstitial flow increases the metastatic potential of breast cancer cells. By varying the concentration of chemoattractants, we decoupled the mechanisms that provide the migratory stimulus and the directional stimulus to migrating breast cancer cells in the presence of a flow field. We found that cells migrated along streamlines in the presence of flow and that the strength of the flow field determined directional bias along the streamline. Furthermore, we validated the autologous chemotaxis model for preferential cell migration in the direction of flow, and we postulate that paracrine signaling leads to migration against the flow.

## MICROFLUIDIC DEVICE DESIGN

We developed a microfluidic cell culture system to investigate the effect of interstitial flow on cells seeded in a 3D matrix (6). The small volumes and length scales of our device allow establishment of molecular gradients in conjunction with flow, and the device geometry allows time-lapse imaging to observe the dynamics of the cell response. The system consists of two channels separated by a region containing cells suspended in collagen I gel. By applying a hydrostatic pressure gradient across the gel region, a consistent flow field is generated. The geometry of the system allows computational modeling of the flow field, which is crucial for experimental design and data quantification. We validated the flow field by adding fluorescent microspheres to the bulk fluid. These spheres can be tracked using fluorescence microscopy, and in conjunction with streak photography, these tracks can be matched to the predicted flow velocities. We found that the measured velocities were repeatable and agreed with the predicted velocities. Furthermore, we used confocal imaging to validate that the cells were seeded in 3D, and we used reflectance imaging and confocal time-lapse imaging to show that cells migrated in the 3D matrix rather than on the surfaces.

## CELL MORPHOLOGY IN FLOW FIELD

We developed a metric for measuring cellular alignment of MDA-MB 231 breast carcinoma cells in a flow field. Cells were imaged at different time points, and an ellipse was fit to each cell's shape. By taking the dot product of a vector oriented

along the major axis of the ellipse with the local streamline vector, an angle of alignment could be determined. We found that after 48hrs, cells exposed to physiologic flow velocities aligned with the streamlines of the flow field while cells not exposed to flow remained randomly oriented (Figure 1). Furthermore, we observed that cellular migration velocity is a function of epidermal growth factor (EGF) concentration and is independent of flow for fluid velocities less than 20μm/s. However, even in the absence of EGF, the cells aligned with the flow suggesting that the flow imparts a directional stimulus while the EGF imparts a migration stimulus to the cancer cells.

## CELL MIGRATION IN FLOW FIELD

Using time-lapse imaging, we tracked the motion of each cell's center of mass. We compared the resulting migration vectors with the streamlines of the flow and found that flow induces directional bias in migration. Furthermore, we found that this directional bias was a function of flow rate. At a flow rate of 0.2μm/s, we found that >80% of cells migrated along a streamline with 46% of cells migrating upstream and 43% of cells migrating downstream. However, at a higher flow rate of 2.0μm/s, we found that similarly >80% of cells migrated along the streamline, but 53% of cells migrated upstream while only 26% of cells migrated downstream (Figure 2).

With the addition of anti-CCR7 blocking antibody, the downstream migration bias was attenuated, but a higher percentage of cells migrated upstream. These data support the hypothesis by Shields et al. that autologous chemotaxis via CCR7 leads to downstream migration, but the data also suggest that a separate pathway is responsible for the upstream migration bias.

Because the flow field washes secreted molecules downstream, cells can only detect paracrine signals secreted by cells located upstream. By reducing the cell concentration in the gels, we can effectively increase the intercellular distance and consequently decrease the strength of paracrine signal concentration field. We found that when cell concentration is decreased, fewer cells migrated upstream, but a higher percentage of cells migrated downstream (Figure 2). These data suggest that upstream migration is a result of paracrine signaling. In summary, we demonstrated that interstitial flow provides a directional stimulus to cancer cell migration, possibly through competing autocrine and paracrine pathways.

## ACKNOWLEDGMENTS

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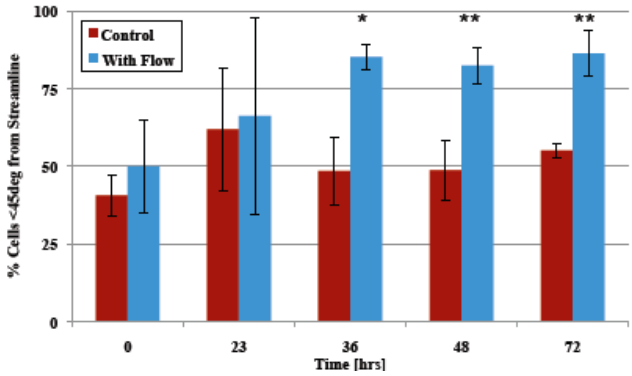


**FIGURE 1. ALIGNMENT OF CELLS TO FLOW STREAMLINES OF VELOCITY FIELD WITH MEAN VELOCITY 2μm/s (* p<0.05, ** p<0.01).**

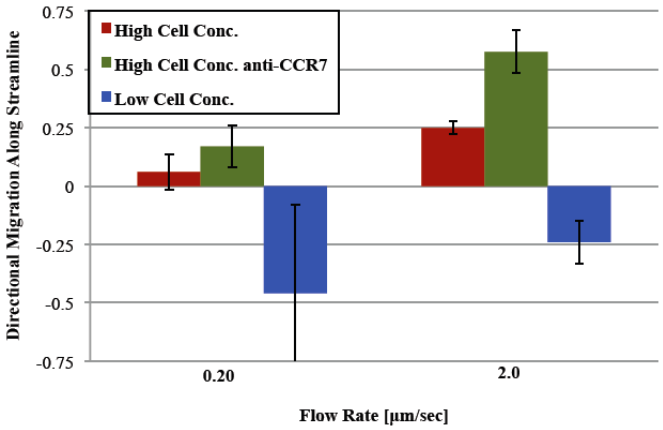


**FIGURE 2. AVERAGE DIRECTIONAL SCORE FOR MIGRATION OF CELLS ALONG STREAMLINE. CELLS MIGRATING AGAINST THE FLOW WERE SCORED WITH +1, AND CELLS MIGRATING WITH THE FLOW WERE SCORED WITH -1. CELLS MIGRATING AT AN ANGLE GREATER THAN 45deg FROM STREAMLINE WERE NOT SCORED.**