# Understanding the Molecular Mechanism of Gradient Sensing

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and torque with the formation of a large, flat contact area in low-viscosity cells leads to a dramatic decrease in the bond force and stable rolling. VECAM is able to reproduce the velocities and shape changes of rolling cells measured in vitro and in vivo.

**1633-Pos Board B525**
**Modeling Killer Cell Migration and Search Strategies**
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During the immune response, cytotoxic T lymphocytes (CTL) and Natural Killer cells (NK cells) patrol the human body. Upon recognition of virus-infected or cancerous target cells, the killer cells release lytic granules at the immunological synapse and kill the targets. For optimal immune function, the migration machinery has to be adjusted in order to maximize the search efficiency.
We used in vitro time lapse microscopy on primary human CTL and NK cells to study their migration in a two-dimensional geometry. In the absence of target cells, CTL alternate between a mobile and a stationary state. The preference for each state depends on the external calcium concentration. On a short time scale (seconds/minutes), killer cell migration is characterized by directional persistence, while migration on a longer time scale (minutes/hours) is random. A mathematical model of a persistent random walker can accurately describe killer cell migration and reveals an optimum for target search efficiency. This optimum depends on the length of persistent motion in one direction and the size of the search space. We believe that this model is very useful to understand how regulation of killer cell migration can serve to optimize the immune response.

**1634-Pos Board B526**
**Analyzing the Early Tissue Mechanical Response to Chemokine Signaling using Microfluidics**
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Directed cell sheet migration is an important process that sculpts the shape of an organism and its internal tissues during development. The patterning of cell sheets and their morphogenetic movements are regulated by both internal genetic programs and external cues. The fate of tissues and their ultimate physiological function relies on integrating genetic programs and processing external signals to provide positional information to guide migration and induce cell differentiation. Considerable effort has been focused on the induction of gene expression through signaling pathways and how tissue architecture can be remodeled however many pathways also regulate the cytoskeleton. Considering the important role cell mechanics plays in tissue self-assembly little is known about the mechanical response of the tissues to chemical cues. One of the reasons of this knowledge gap is the lack of the technologies to analyze mechanical responses to chemokine signals in vivo. Our group has focused on developing novel microfluidic devices and understanding the dynamic responses of intact epithelial tissues to acute chemical stimulation. We have characterized the dynamic response of a glucocorticoid receptor family biosensor to periodic dexamethasone stimulation (Kim et al, ) and the response of a G-protein coupled receptor family to spatiotemporal patterns of ligand stimulation (Kim et al, in preparation). Here, we demonstrate a rapid mechanical response to tyrosine kinase receptor activation by soluble fibroblast growth factor (FGF). We are able to change FGF at the surface of an early Xenopus leavis embryonic tissue in a spatiotemporally controlled way using a custom designed microfluidic channel with laminar flow interfaces. using this device we can control the FGF gradient while simultaneously modulating FGF activity through receptor inhibitors. Our work will contribute to advancements in areas including growth factor signaling, spatiotemporal biological control and developmental biology.

**1635-Pos Board B527**
**Understanding the Molecular Mechanism of Gradient Sensing**
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The G-protein coupled receptor CXCR4 plays a central role in stem cell homing, organogenesis, inflammation and tissue repair. It mediates cell migration towards increasing concentration of the chemokine ligand CXCL12 (chemotaxis). The expression of CXCR4 on malignant cells may influence the biology of cancer, in modulating tumor survival, growth and angiogenesis. CXCR4 also plays an important role in directing metastatic CXCR4-expressing tumor cells to organs that express CXCL12. The CXCR4/CXCL12 pathway mediating cell migration is well known and understood. On the other hand the mechanism of gradient sensing remains to be discovered.
Here we use Ewing's sarcoma derived cells to unravel the processes that are involved in CXCR4 related metastasis formation. Cells are transfected with DNA encoding CXCR4-eYFP. using single-molecule imaging technique we measure the diffusion coefficient of the individual receptors in the membrane and analyse diffusion in terms of confinement. We observed a decrease in mobile receptor fraction under global stimulation with the ligand - CXCL12 - in a concentration-dependent manner. In an integral approach, these molecular insights are complemented by whole cell behavior characterization, like motility as obtained from single-cell migration assays. The results are compared with models established for non-cancer cell lines. Our findings highlight the molecular machinery of chemokine gradient recognition and its effect on cell motility in the context of cancer spreading.

**1636-Pos Board B528**
**Fibronectin Induces Beta2-Integrin-Mediated Neutrophil Haptokinesis Independent of Chemoattractant**
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Neutrophils are mediators of innate immunity and inflammation and motility is critical to their function. The relationship between the presentation and identity of adhesive ligands and the dynamics of neutrophil motility is facilitated by the ability to manufacture well-defined adhesive surfaces that microcontact-printing affords. Here we show that by presenting fibronectin (FN) on poly(dimethylsiloxane) (PDMS) substrates, neutrophils are motile. This motility is haptokinetic and does not require prior or co-stimulation by chemoattractant. On printed fields of FN, the motility is phenotypically reminiscent of keratocytes and dependent upon the $\alpha_M\beta_2$ (Mac-1) integrin receptor. The phenotype observed contrasts with the classical hourglass morphology of neutrophils on FN adsorbed to glass. The addition of exogenous formylated chemoattractant increased the basal FN-induced motility in a dosage-dependent manner only when the FN concentration was below a critical threshold. These results suggest that neutrophils are capable of displaying multiple modes of motility as dictated by their adhesive environment. The exquisite cell-FN specificity demonstrated of neutrophils on printed PDMS makes the technique amenable to the future study of motility on more complex patterns of adhesive ligands as well as traction force mapping via micropost arrays.

**1637-Pos Board B529**
**T Lymphocyte Receptor-Ligand Interactions for Migration on Microcontact Printed Intracellular Adhesion Molecule-(ICAM)-1 Surfaces**
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Recruitment of T lymphocytes into lymphoid organs and peripheral tissues during immune surveillance and inflammation is dependent upon a tightly regulated multistep adhesion cascade. Upon endothelium exposure to certain cytokines, cell adhesion molecules are upregulated providing a means for T lymphocytes to tether, roll, adhere, and migrate. Upon binding of the integrin lymphocyte function-associated antigen 1 (LFA-1) to the ligand, intracellular adhesion molecule 1 (ICAM-1), ''outside-in'' signaling is primed to induce firm adhesion and migration. Here, through the use of microcontact printing (μCP) we have studied the random motility of human peripheral T lymphocytes and the effects of the homeostatic chemokines CCL19, CCL21, and CXCL12 on ICAM-1 coated surfaces. Studies have demonstrated that the LFA-1/ICAM-1 ''outside-in'' signaling is in concert with the ''inside-out'' signals produced downstream from chemokine binding to promote chemokinesis. The random motility coefficient (μ) on ICAM-1 surfaces was found to be 200.37 ± 13.30 μm2/min. Unexpectedly, the random motility coefficient had no significant differences between chemokine concentrations even around the KD of the receptors CCR7 and CXCR4. We hypothesize that the ''outside-in'' signaling dictated by LFA-1/ICAM-1 binding overwhelms the ''inside-out'' signaling from the chemokines preventing a difference in the random motility coefficient to be observed. In addition, microcontact printing will permit us to pattern ICAM-1 to observe T lymphocytes on controlled substrates. Furthermore, through the use of a microfluidic device we can begin to elucidate the effects of chemokine gradients on directed T lymphocyte migration. By producing single and competing chemokine gradients, we can calculate the chemotactic index to assess the potency of these chemokines on chemotaxis.