Neutrophil Kinesis on Fibronectin-Printed PDMS and a Biophysical Interpretation

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Motivation

**Improved homogeneity on printed PDMS**

- FN Printed PDMS
- FN Adsorbed Glass

...despite similar protein deposition

Goal

Establish baseline motility metrics for neutrophil haptokinesis and chemokinesis on continuous fields of FN-printed PDMS.

Methodology

**Microcontact Printing**

- Ink
- Print
- Dry

**Cell Tracking**

- 40 µm
- (min)

Results

**Exquisite Cell-FN Specificity**

- Glass BSA
- PDMS F127

No off-FN adhesion observed on printed PDMS, blocked with Pluronic F127.

**Integrin-Mediated Adhesion**

- (+) Control
- (-) Control

Functional antibody blocking revealed Mac-1 (α<sub>5</sub>β<sub>2</sub>) was an integrin receptor mediating FN adhesion.

**L-Selectin as Activation Marker**

- 488-Granzyme
- 555-TNF
- 647-Pro-FN

An active phenotype (i.e. low L-Selectin) was not found prior to FN exposure, suggesting binding and subsequent motility were FN-induced via outside-in

A Model Independent Motility Analysis

- Haptokinesis
- Chemokinesis

Extent of haptokinesis ("No fMLF") is constant over FN range tested. Yet, during chemokinesis, fMLF only increases motility below an adhesive threshold.

Superdiffusive Motility

Neutrophils accumulate squared displacement superdiffusively. Dotted lines are best-fits to descriptive power-law model: MSD(τ) = A<sup>τ</sup>.

Summary

- Printed FN on PDMS elicits homogeneous neutrophil population
- Adhesion is Mac-1 (α<sub>5</sub>β<sub>2</sub>) mediated
- Adhesion and haptokinesis are induced via an outside-in integrin activation pathway
- Cells are dynamically altering either bond number or affinity state to achieve constant haptokinesis
- Kinesis is superdiffusive

Looking Forward

**Correlating Discrete Force Fluctuations with Whole-Cell Trajectories**

Hypothesis: Cell kinesis is the manifestation of an ensemble of random walks at the single motor (myosin) lengthscale.

Continuous Fields to Discrete Islands

Hypothesis: Cells integrate adhesive contact across entire cell body. This will manifest itself in motility metrics being similar on continuous fields and discrete islands if ligand density per total contact area is preserved.

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