Neutrophil Kinesis on Fibronectin-Printed PDMS and a Biophysical Interpretation

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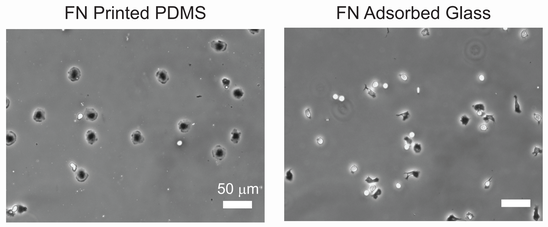
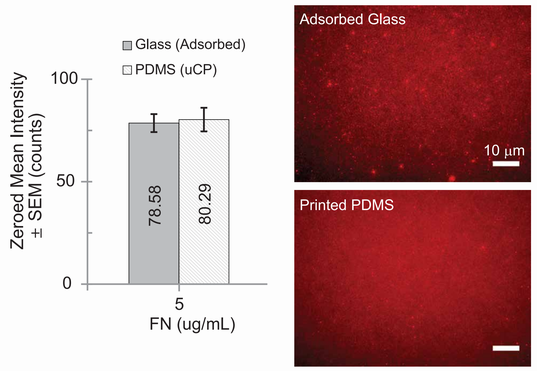
Motivation

Improved homogeneity on printed PDMS



...despite similar protein deposition

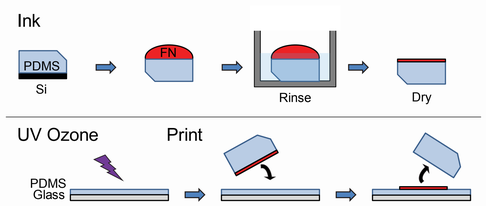


Goal

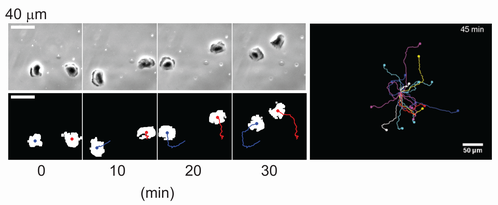
Establish baseline motility metrics for neutrophil haptokinesis and chemokinesis on continuous fields of FN-printed PDMS.

Methodology

Microcontact Printing

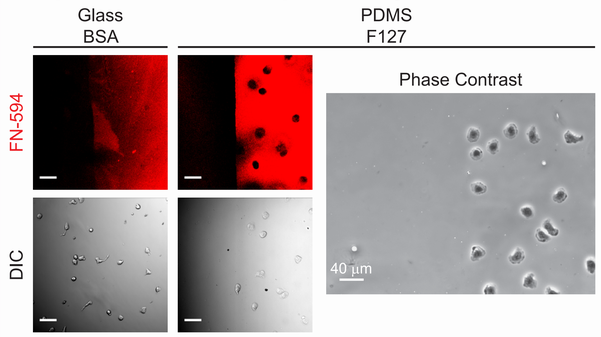


Cell Tracking



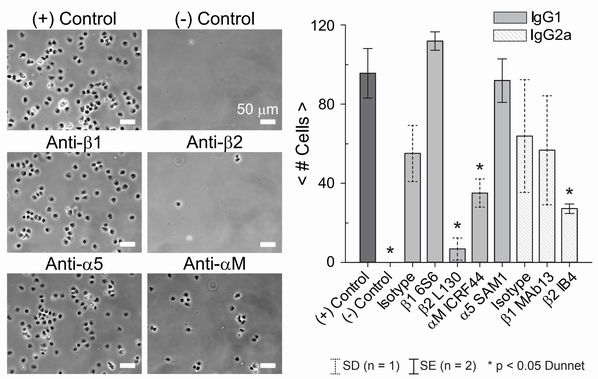
Results

Exquisite Cell-FN Specificity



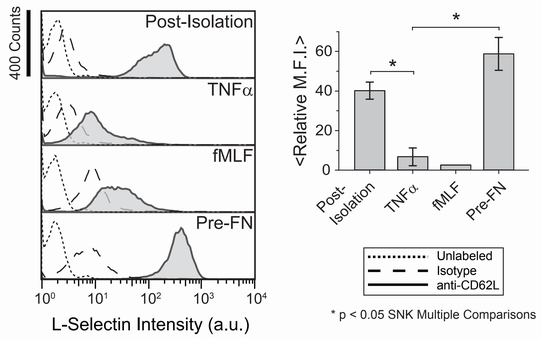
No off-FN adhesion observed on printed PDMS, blocked with Pluronic F127.

Integrin-Mediated Adhesion



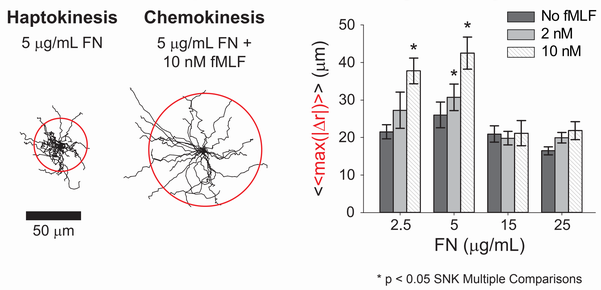
Functional antibody blocking revealed Mac-1 ($\alpha_M\beta_2$) was integrin receptor mediating FN adhesion.

L-Selectin as Activation Marker



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

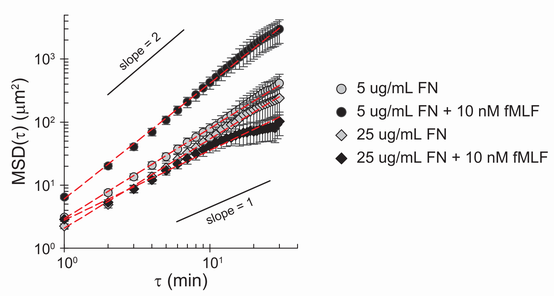
A Model Independent Motility Analysis



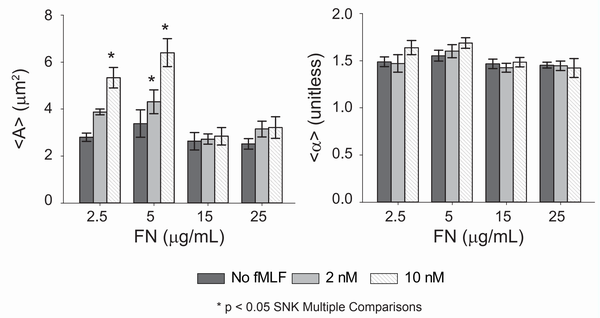
* p < 0.05 SNK Multiple Comparisons

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: $MSD(\tau) = A\tau^{\alpha}$.



* p < 0.05 SNK Multiple Comparisons

Trend previously revealed is captured in best-fit parameter A defined a MSD(τ = 1 min). Across all conditions tested best-fit power law exponent α is relatively constant with superdiffusive value ~ 1.5.

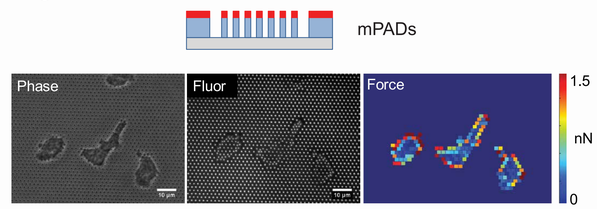
Summary

- Printed FN on PDMS elicits homogeneous neutrophil population
- Adhesion is Mac-1 ($\alpha_M\beta_2$) 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

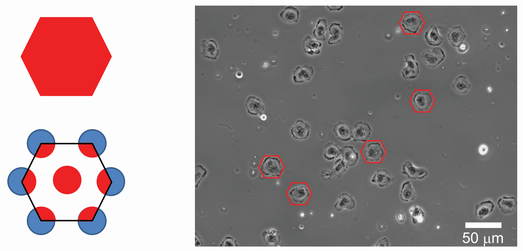
Correlating Discrete Force Fluctuations with Whole-Cell Trajectories

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.



Acknowledgments

Gratitude is expressed to Christopher S. Chen, PhD and his students for sharing μCP and mPADs expertise.

Funding provided by NIH (HL18208) to DAH and NSF Graduate Research Fellowship to SJH.