Fibronectin Induces β_2-Integrin-Mediated Neutrophil Haptokinesis Independent of Chemoattractant

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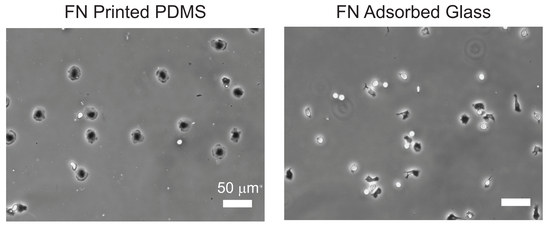
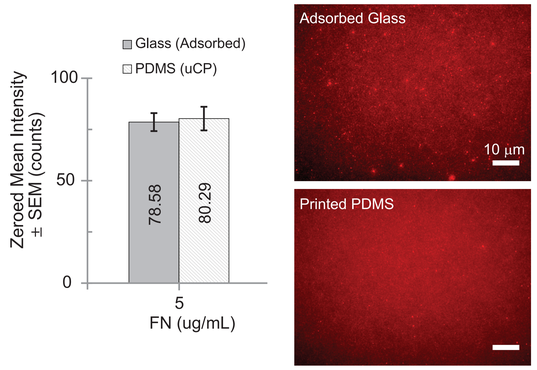
Motivation

Improved homogeneity on printed PDMS



...despite similar protein deposition

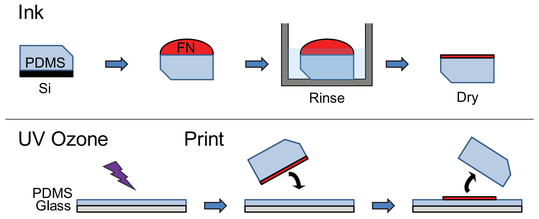


Goal

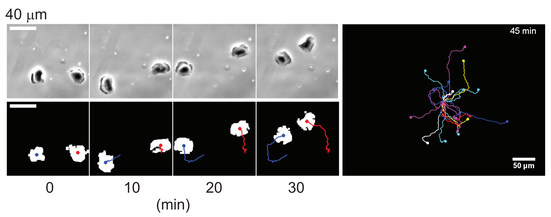
Establish baseline motility metrics for neutrophil haptokinesis and chemokinesis on continuous fields of FN-printed PDMS.

Methodology

Microcontact Printing

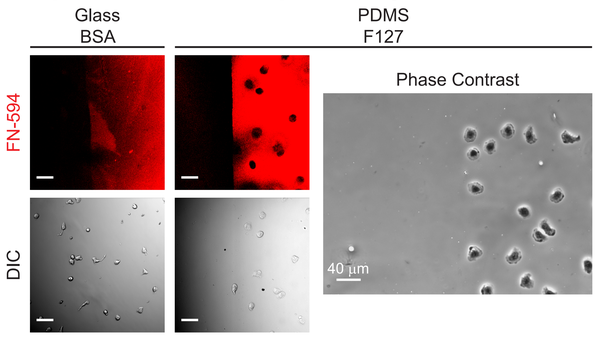


Cell Tracking



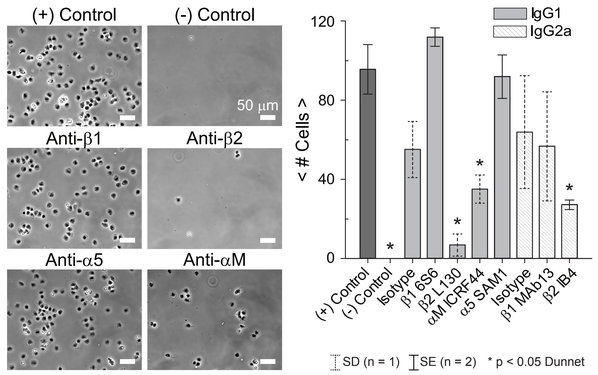
Results

Exquisite Cell-FN Specificity



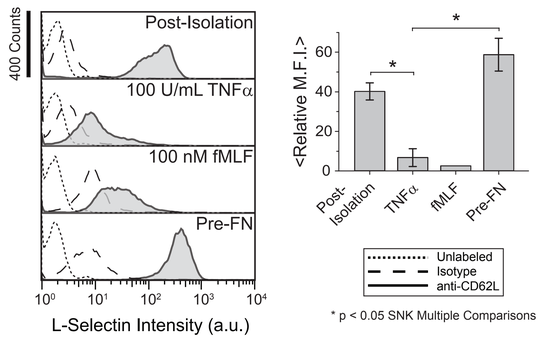
No off-FN adhesion observed on printed PDMS, blocked with Pluronic F127.

Integrin-Mediated Adhesion



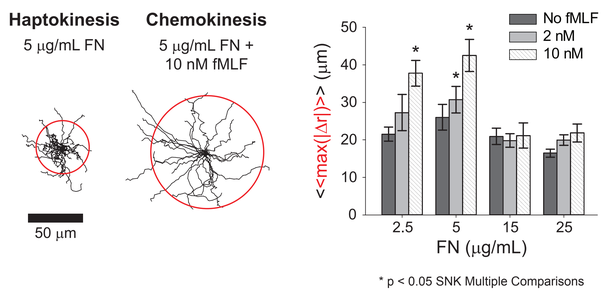
Functional antibody blocking revealed Mac-1 ($\alpha_M\beta_2$) integrin receptor mediated adhesion to FN on PDMS.

L-Selectin as Activation Marker



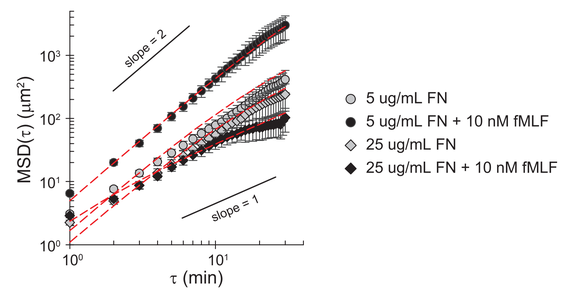
An active phenotype (i.e. low L-Selectin) was not found prior to FN exposure, suggesting binding and subsequent motility were FN-induced via an outside-in pathway.

Model Independent Motility Analysis

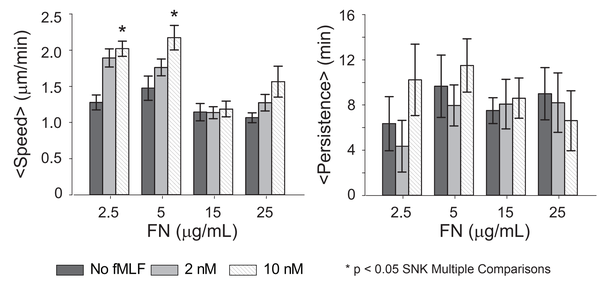


Extent of haptokinesis ("No fMLF") was constant over FN range tested . During chemokinesis, fMLF only increased motility below an adhesive threshold.

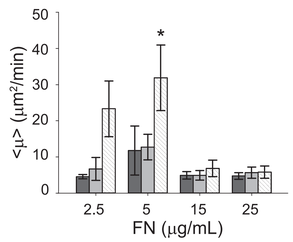
Persistent Random Walk Model



Dotted lines are best-fits to persistent random walk model: $MSD(\tau) = 2S^2P[\tau - P(1-\exp(-\tau/P))]$.

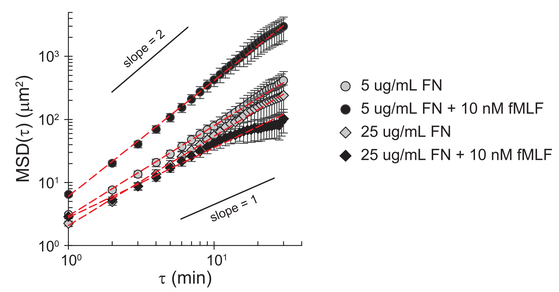


Majority of variation was seen in mean cell speed.



Computation of the random motility coefficient ($\mu = S^2P/2$), reveals the trend previously observed in the model-independent analysis.

Power-Law Model



Dotted lines are best-fits to a simple power-law model: $MSD(\tau) = A\tau^{\alpha}$. Neutrophils accumulated squared displacement superdiffusively.

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

Summary

- Printed FN on PDMS elicits homogeneous neutrophil population
- Difference in morphology on glass vs. PDMS is attributed to protein conformation not content
- Adhesion to FN on PDMS is Mac-1 ($\alpha_M\beta_2$) mediated
- Motility is induced via an outside-in integrin activation pathway
- Constant basal motility induced by FN is sub-maximal
- Both kineses are superdiffusive

Acknowledgements

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