Cell Migration, the Cytoskeleton, Chemotaxis, and Haptotaxis

3/9/17
ChE 575
When, Where, Why do cells migrate?

1. Neutrophil Migration to Battle Infection
2. Development
3. Wound Healing
4. Disease
Wound Healing
Disease

Jeon et al. 2014
Basic Migratory Process Observed through Time-Lapse microscopy
Cells connect to the ECM: 
ECM $\rightarrow$ Integrin $\rightarrow$ Focal Adhesion $\rightarrow$ Actin

Transmit force and movement in cell via cytoskeleton and focal adhesions.
Tension is translated to biochemical information at adhesion sites

FRET: Fluorescence (Forster) Resonance Energy Transfer

P = Protruding
R = Retracting

Grashoff and Hoffman et al. 2010
Actin filaments: double helix with 5-9nm diameter, connect to integrins (indirectly via focal adhesion proteins)
Each class of filaments is a polymer:
- made up of smaller, soluble subunits

Cells using ATP energy to polymerize and depolymerize monomers when needed
Electron Micrograph view of the Actin cytoskeleton in Lamellipodia

Michele Balsamo & Leslie Mebane, Gertler Lab, MIT
Catch vs. Slip bonds

Slip Bonds

\[ k_{\text{off}}(f) = k_{\text{off}}^{\circ} \exp(x \beta f/k_B T) \]

Catch-Slip Bonds:
Calculating rupture force as a function of loading rate

\[ f^* = \frac{k_B T}{x \beta} \ln\left(\frac{x \beta}{k^0 k_B T}\right) + \frac{k_B T}{x \beta} \ln(r_f). \]

\( \chi_B \) Characteristic Bond Length

\( k^0 \) Unloaded Dissociation Rate Constant

\( r_f \) Rate of application of force

Guo and Guildford, 2006
Let’s look at movement more closely – how do we measure/predict?
Sample Movies from Peyton Lab

Breast Cancer Cells migrating on a biomaterial
Courtesy Peyton Lab
How does one quantify this movement?

**Speed**

$$Speed(t_1 - t_2) = \frac{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}}{(t_2 - t_1)}$$

$$TotalSpeed = \frac{\sum_{i} Speed}{\# time intervals}$$

**Displacement**

$$displacement = \sqrt{(x_f - x_i)^2 + (y_f - y_i)^2}$$

**Path Length**

$$PathLength = \sum \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$
Mean Squared Displacement analysis
Free diffusion

\[ \langle r^2 \rangle = 2NDt \]

Dimension (1, 2 or 3)
Diffusion coefficient
Migration is Random at Long Time points, but persistent at short intervals

Longer timepoints (min-hr):
Cell locomotion observed

Breast Cancer Cells migrating on a biomaterial
Courtesy Peyton Lab
Accounting for this in MSD analysis
Persistent Random Walk

\[ \langle r^2(t) \rangle = 2S^2 P(t - P + Pe^{-t/P}) \]
<table>
<thead>
<tr>
<th>Cell Type</th>
<th>$P$ (min)</th>
<th>$S$ (μm/min)</th>
<th>$\mu$ ($cm^2/s$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>1–4</td>
<td>20</td>
<td>$30 \times 10^{-9}$</td>
</tr>
<tr>
<td>Macrophages</td>
<td>30</td>
<td>2</td>
<td>$10 \times 10^{-9}$</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>60</td>
<td>0.5</td>
<td>$1.2 \times 10^{-9}$</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>300</td>
<td>0.4</td>
<td>$6.2 \times 10^{-9}$</td>
</tr>
<tr>
<td>Smooth muscle cells</td>
<td>240–300</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Neurons on laminin [74]</td>
<td></td>
<td>1–3</td>
<td></td>
</tr>
<tr>
<td>Cerebellar granule cell neurons</td>
<td>Saltatory</td>
<td>~ 1</td>
<td></td>
</tr>
<tr>
<td>migrating on astroglial fibers [75]</td>
<td></td>
<td>(with pauses and long breaks)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05–0.3</td>
<td>(when observed over several hours)</td>
</tr>
<tr>
<td>Cerebellar granule cell neurons</td>
<td>Saltatory</td>
<td>~ 0.1</td>
<td></td>
</tr>
<tr>
<td>migrating on laminin-coated or</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>astroglial membrane-coated glass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fibers [76]</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The variables are defined in the text: $P$ is the persistence time, $S$ is the speed of migration, and $\mu$ is the random motility coefficient.
Anomalous diffusion: Often confined

If there are obstacles or traps in the way, diffusion might be anomalous (depends on obstacle concentration).

\[ \langle r^2 \rangle = 2NDt \]

\[ \langle r^2 \rangle = 2ND\tau^\alpha \]
What causes directed migration? (Haptotaxis)

- Soft
  - Low Growth Factor
  - Single Cell
  - Downstream in shear flow
- Stiff
  - High Growth Factor
  - Along Cell Tracks
  - Upstream in shear flow

- Duro
- Chemo
- Plitho
- Rheo
Haptokinesis vs Haptotaxis

DiMilla et al., JCB 1993

Increasing Protein Concentration
(FN or Collagen IV)
The phenomenon

The most significant finding in this study is that cultured cells can guide their movement by probing the substrate rigidity. As the leading edge crosses onto rigid substrates, lamellipodia and lamella expand, leading to directed migration onto the rigid substrate. Conversely, as the leading edge approaches the soft side, local retractions take place, causing the cell to change direction.

In addition to substrate rigidity, we have demonstrated that mechanical input generated by substrate deformation also regulates the formation and retraction of lamellipodia. This is to be expected in an active sensing system, because the force/deformation caused by the external manipulation will be superimposed on the effects of the cellular probing forces. In all cases cells responded with the formation/expansion of lamellipodia when the substratum was locally pulled outward from the center, and with retraction when the substratum was pushed inward. Because fibroblasts ex-
Durotaxis: gradients via photomask polymerization

Wong, J. Langmuir, 2003
Adapting microfluidics to create haptotactic gradients

Burdick et al., Langmuir 2004
Durokinesis: Biphasic Migration Dependence on Substrate Stiffness

- Durokinesis: SMCs migrate fastest on an ‘optimally stiff’ substrate
- Actin polymerization controlled by adhesive protein density as well (Haptokinesis).
- Cells need stiffer substrate when less fibronectin is attached to surface to migrate at maximum capacity

Cytoskeletal Assembly Regulated by Substrate Stiffness

Chemotaxis: Controlling Direction of Motility via Soluble Chemical Cues
Chemotactic Index is a measure of how efficiently a cell follows a chemical gradient.

\[ C.I. = \frac{\text{Displacement (\(\mu m\))}}{\text{PathLength (\(\mu m\))}} \]

\[ 0 \leq C.I. \leq 1 \]
In vitro Chemotaxis

Boyden Chamber

Under-Agarose Assay

Microfluidics
Plithotaxis: Cells Migrate in the Direction of the Greatest Normal Stress and Lowest Shear Stress
Rheotaxis: Cell Migration Upstream in Shear Flow

Polacheck et al. 2014
Mechanotransduction

• The ability of a cell to turn a mechanical cue from the ECM into an intracellular signal
  – RhoA, pSrc, pAkt

• And eventually into a phenotypic response
  – Migration, differentiation, shape, growth
Mechanotransduction: Cell can translate Mechanical Information from the ECM to an intracellular biochemical signal
How does this happen?

• Focal adhesions.
  – Remember, those connections between integrins and the actin cytoskeleton in a cell.

• When, how do focal adhesions re-arrange in response to mechanical forces?
Vibrating Cells: Cells will pull at the site of vibration

http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0026181#s5
Pulling on cell attachment points: Focal adhesions are recruited to the site of stretch
Stretching the underneath substrate: Microtubules assemble (polymerize) when cell is stretched

Putnam et al., JCS, 1998
Proposed: Cell-ECM force balance through F-actin and microtubules

- In response to extracellular stretch or an intrinsic ECM stiffness, F-actin microfilaments adjust in tensional resistance, and the microtubule network adjusts in compressive resistance.
Tensegrity: a Physical Mechanism of Mechanotransduction

Cytoskeleton connects from focal adhesions to nucleus. Forces at focal adhesions can propagate to changes in shape of nucleus → affects transcription regulators → gene expression/phenotype

Long-distance propagation of forces in a cell

Ning Wang, Zhigang Suo

Biochemical and Biophysical Research Communications 328 (2005) 1133–1138
Migration Through Small Channels Causes Nuclear Strain and Rupture

Denais et al. 2016
McGregor et al. 2016
Modeling of Nuclear Mechanics that Limit Cell Motility

\[ \sigma_{\text{von Mises}} \]

\[ \sigma_y \]

\[ \frac{\sigma_{\text{von Mises}}}{\sigma_y} \]

\[ \begin{align*}
\frac{\sigma_{\text{von Mises}}}{\sigma_y} & = \frac{\frac{1}{2} (\sigma_1 - \sigma_2)^2 + \frac{1}{2} (\sigma_2 - \sigma_3)^2 + \frac{1}{2} (\sigma_3 - \sigma_1)^2}{\sigma_y} \\
& = \frac{\sqrt{\left(\frac{1}{2} (\sigma_1 - \sigma_2)^2 + \frac{1}{2} (\sigma_2 - \sigma_3)^2 + \frac{1}{2} (\sigma_3 - \sigma_1)^2\right)^2}}{\sigma_y}
\end{align*} \]

Cao et al. 2016
Tension Alters Gene Expression

Tajik et al. 2016
Traction Force Microscopy: Tool to Measure Cellular Forces Exerted on Substrate
Mechanical regulation of cell function with geometrically modulated elastomeric substrates

Jianping Fu¹⁻³, Yang-Kao Wang¹⁻³, Michael T Yang¹, Ravi A Desai¹, Xiang Yu¹, Zhijun Liu¹ & Christopher S Chen¹

NATURE METHODS | VOL.7 NO.9 | SEPTEMBER 2010
Have a Good Break!

• Reminder: You have a paper review on Tuesday after break