Microsystems to study leukocyte chemotaxis – from gradient generators to micropatterned surfaces

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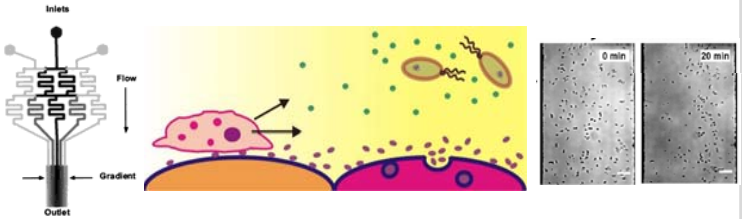
Merida, 14.12.10

Institute of Organic Chemistry (IOC)



Overview

- Cell-based applications of MEMS
- The importance of cell migration
- Traditional migration assays
- Gradient generators
- Microstructured surfaces
- 3D chemotaxis assays
- Outlook

Advantages of Miniaturization

- Reduction of total sample volume (ml → nl)

⇒ **Cost effectiveness**

- Maximization of usable surface area for reaction/interaction

⇒ **Acceleration**

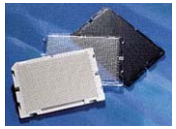
‘‘lab-on-a-chip’’:

- low volume liquid handling devices + biochip platforms
- Sequential steps performed in one device
- High extent of **parallelization**

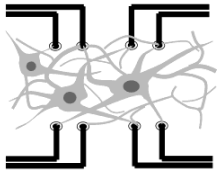
- Cell sized-devices to observe **individual cell responses**

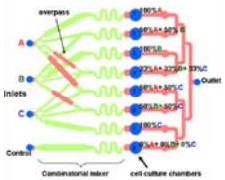
Cell-based assays in microsystems

- High-througput cell-based screens
- Cell-based Biosensors
- Monitoring cell behaviour in microfludic devices



1536well / 3456well plates





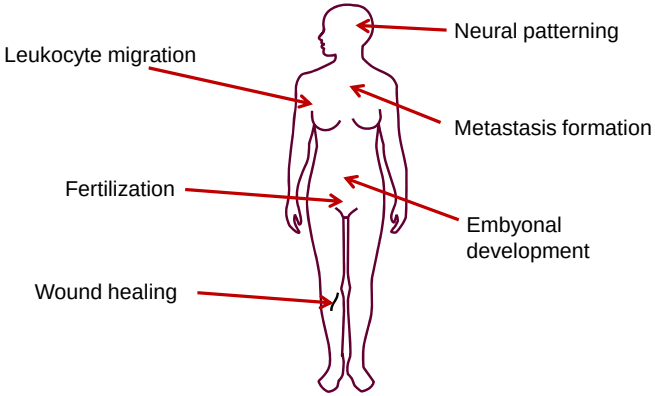
Monitoring cellular behavior

- Bacteria: Biofilm formation, chemotaxis
- Fungi: Dyctiostelium discoideum behavior
- Plants: Settling of algae,
- Animals: stem cell differentiation, tumor development

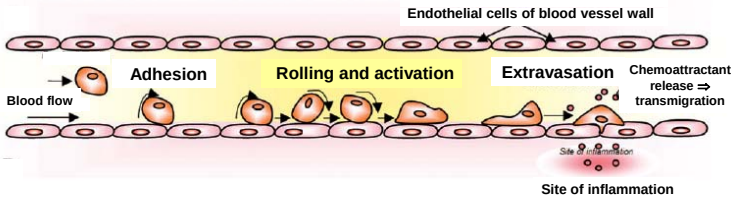
bacteria

slime mold

Cell migration in humans

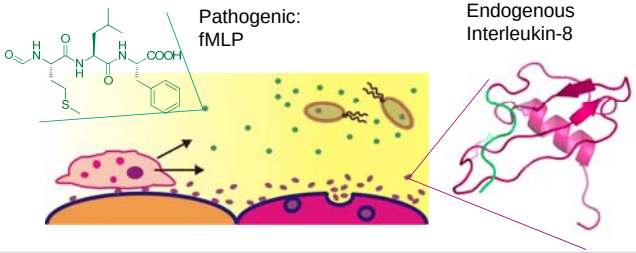


Chemotaxis in inflammatory processes



Clues that influence leukocyte chemotaxis

- Concentration of chemoattractant
- Gradient of chemoattractant
- Mix of chemoattractants
- Surrounding matrix (elasticity, composition…)
- Blood flow (shear forces)



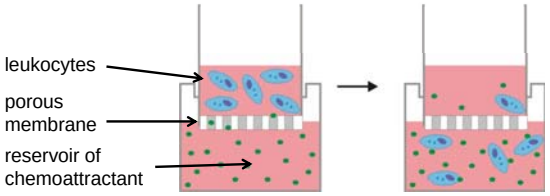
Leukocytes are good to observe:

Challenges in the design of chemotaxis assays

- Chemoattractant gradient
  - Stability over time
  - Stability in space
  - Defined profile
  - Multi-compound gradients
- Realistic setting
  - Lining of the walls with cell adhesion molecules
  - Elasticity of the medium
  - Shear stress on cells
  - Mimicking extravasation (blood-vessel → tissue)
- channel size, flow speed, temperature, cell loading…

Traditional approaches: Boyden chamber (1962)

Transwell Assay



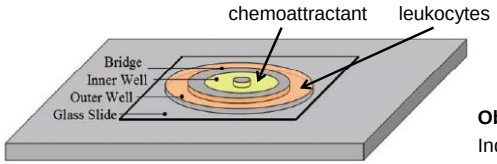
Count:
cells in insert
cells in reservoir

+ easy quantitative evaluation

- chemokine gradient is not stable ⇒ time dependence
- large numbers of cells required
- no single cell responses
- no well defined gradient

Boyden, *S. J. Exp. Med.,* 1962

Traditional approaches: Dunn chamber (1991)



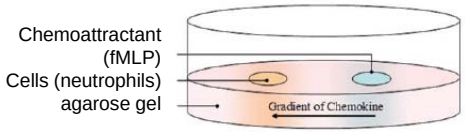
Observe:
Individual cells on „bridge“

+ stable over several hours
+ predictable gradient behavior
+ reproducible thickness of gradient layer

- no fast gradient changes
- only linear gradients

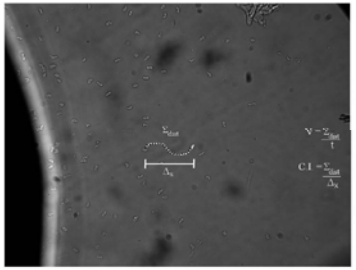
Zicha, J. *Cell Sci,* 1991

Traditional approaches: Agarose assay



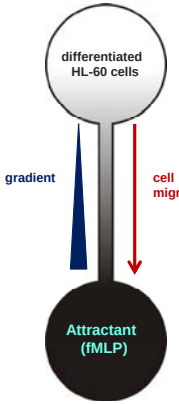
+ stable over several hours
+ 3D environment

- gradient takes long to form
- no fast gradient change
- only linear gradients
- no control over gradient



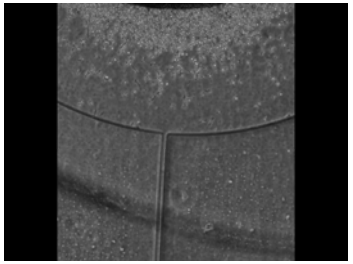
Heit, *J. Cell Sci.*, 2005

Diffusion-based gradients in microchannels



- defined channel geometry
  ⇒ defined chemical gradient
- channel in cellular dimensions
- large reservoirs to maintain gradient

Migration towards channel entrance



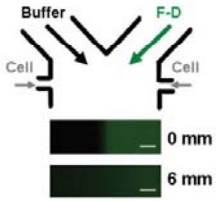
Velocities:
Reservoir:
2.5 µm/min
Channel:
55 µm/min

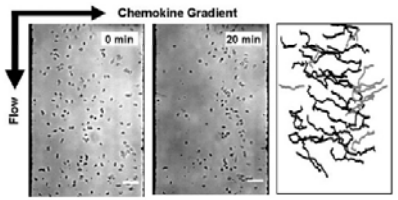
Neutrophils

A. Rosenhahn, University of Heidelberg

‘‘Y’’ type microfluidic gradient generator

Continuous laminar flow for generating gradients





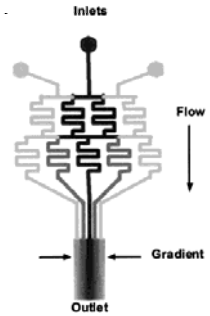
+ rapid evolution of gradient
+ stable gradient in observation region
+ muticompound mixing possible
+ slow flow rate ⇒ low shear stress
  ⇒ little effect on direction

T-cell velocities:
CCL19: 6.2 ±1.0 mm/min
CXCL12: 7.4 ± 0.2 mm/min

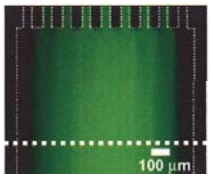
Lin and Butcher, *Lab Chip,* 2006

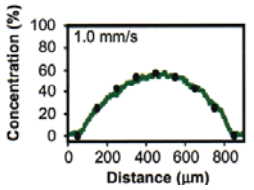
Pyramidal networks for complex gradient shapes

controlled diffusive mixing of laminar flow fluids
by repeated splitting, mixing, and recombination of fluid streams



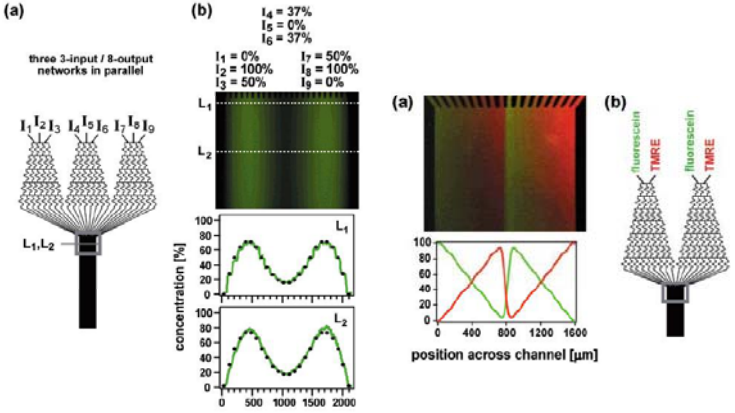
+ steady states
+ dynamic gradients
+ gradients of different profiles and resolution
+ complex gradient shapes





Jeon, *Langmuir*, 2000

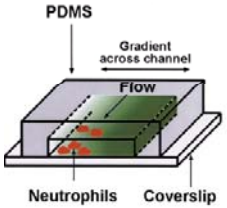
Pyramidal networks for complex gradient shapes

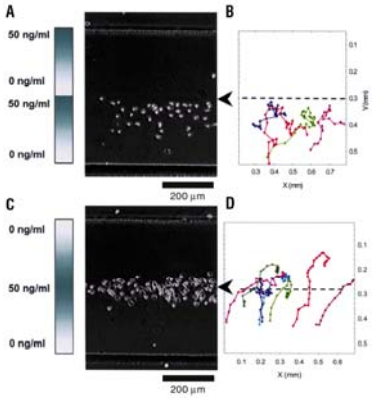


Dertinger, *Anal. Chem*. 2001

Neutrophil response to IL-8 gradient profiles

+ spatially and temprorary controlled gradients
+ single cell tracking
+ quantification of chemotactic flux and chemotactic coefficient
- limit: gradient smearing

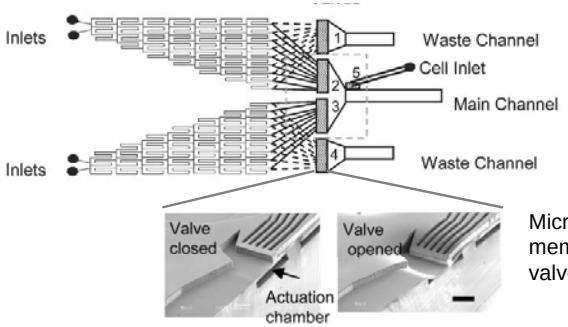




Jeon, Nat. Biotechnol. 2002

Temporal gradient control

Two gradient generating networks

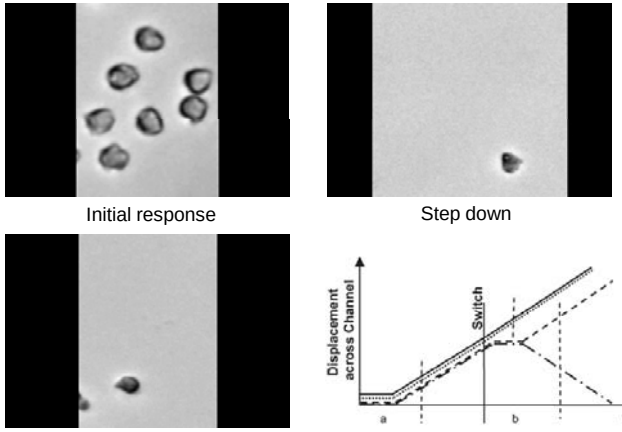


Microstructured membranes as valves

⇒ fast and accurate switches between any two stable gradients

Irimia, *Lab Chip,* 2006

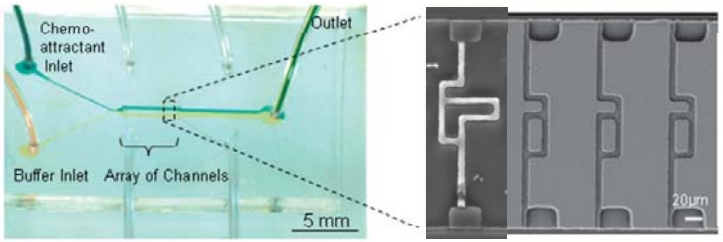
Neutrophil responses to IL8-gradient changes



Irimia, *Lab Chip*, 2006

Non-linear migration tracks

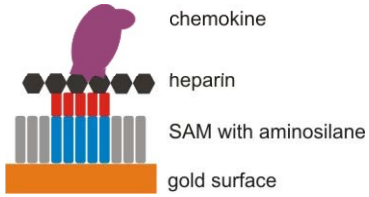
Can leukocytes find the shortest way to the gradient origin?

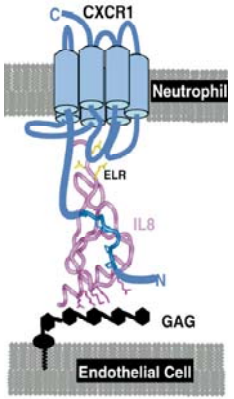


Ambravaneswaran, *Integr. Biol., 2010*

Oriented chemokines in 2D micropatterns

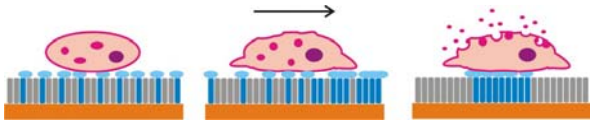
- Hypothesis:
  Binding of chemokines to glycosaminoglycans orients them for improved receptor binding
- Idea: Immobilize chemokines so that receptor binding site faces away from surface



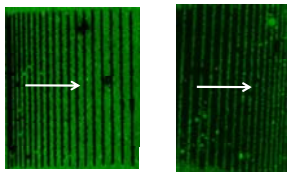


Skelton, *Structure,* 1999

Chemokine surface patterns as 2D-gradients



Even distribution | Gradient pattern | High-density patches

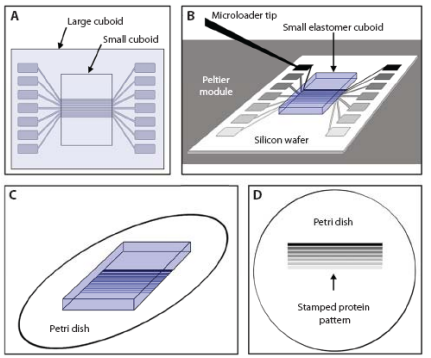


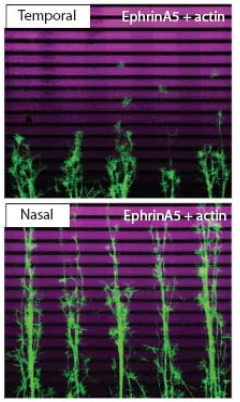
Gradient patterns microcontact printing

Parameters to explore:

- Influence of pattern on response
- Role of background molecules
- Threshold concentration for migration

Microfluidics meets microcontact printing

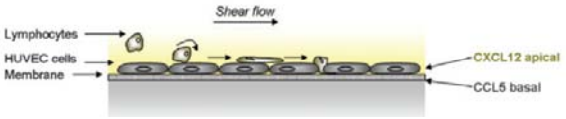




Von Philipsborn, *Science STKE*, 2007

How to make chemotaxis assays more life-like?

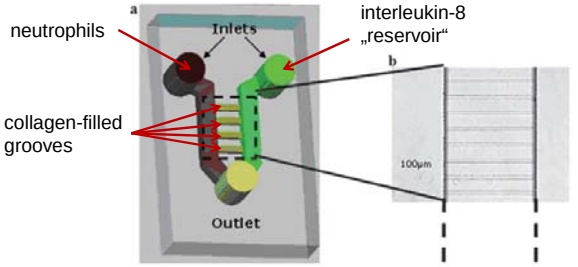
- confluent layer of endothelial cells on coated channel
- continuous flow of cells
- soft gel scaffolds (agarose, ECM-like matrices)
- 3D structures
- chemokine gradient released from tissues through endothelium



Boyden chamber coated with endothelial cells

Toetsch, *Integr. Biol.,* 2009

Ladder Chamber: Gel Chemotaxis & Microfluidics

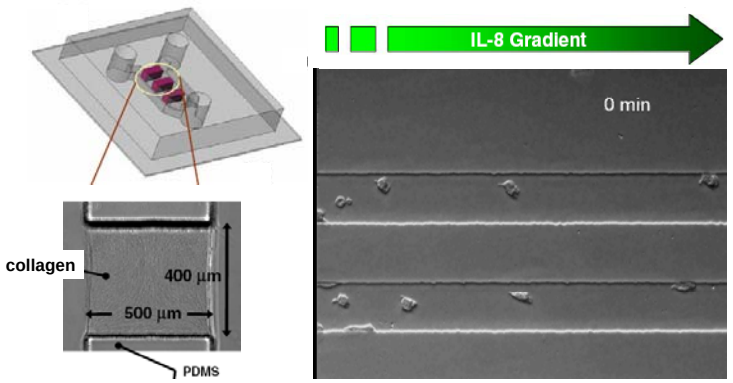


- IL-8 diffuses through collagen matrix
- cells move perpendicular to central flow

⇒ „extravasation“

Saadi, *Biomed. Microdevices*, 2007

Neutrophil migration in the Ladder Chamber



Saadi, *Biomed. Microdevices*, 2007

Summary

- Chemotaxis is important to many biological processes
- Traditional diffusion-based chemotaxis assays may be too simple
- Microfluidic gradient generators for
  - stable gradients of one or two compounds
  - complex profiles
  - temporal control
- Micropatterned surface to study effects of adherent chemoattractants
- Hydrogels as 3D-matrices for migration
- Cell-coated devices as life-like environment

Conclusions and Considerations

- Micro electromechanical systems have brought cell-based experiments to the next level
- More „life-like“ = more parameters to control
- Every biological question poses a new set of challenges
- The suitability of an approach depends on the biological question adressed
- Easy-to-operate systems are more likely to be used in life science

Conclusions and Considerations

- Life scientists and engineers need to communicate:
  - the requirements of biological systems
  - the available technological means