Chirotaxis: matrix chirality modulates the cell migration speed

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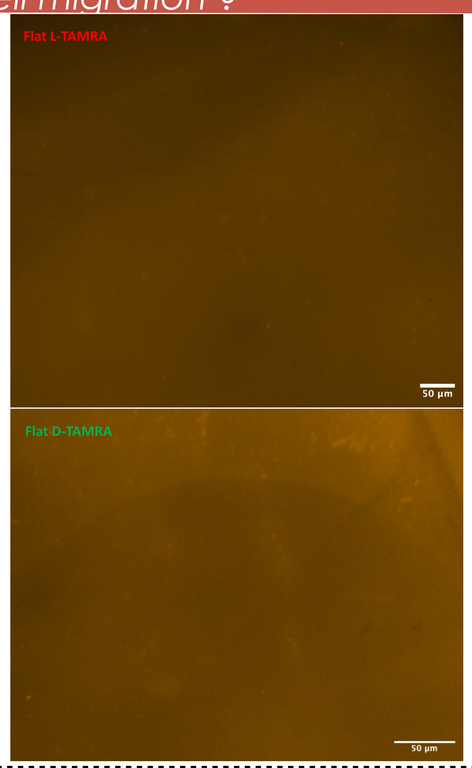
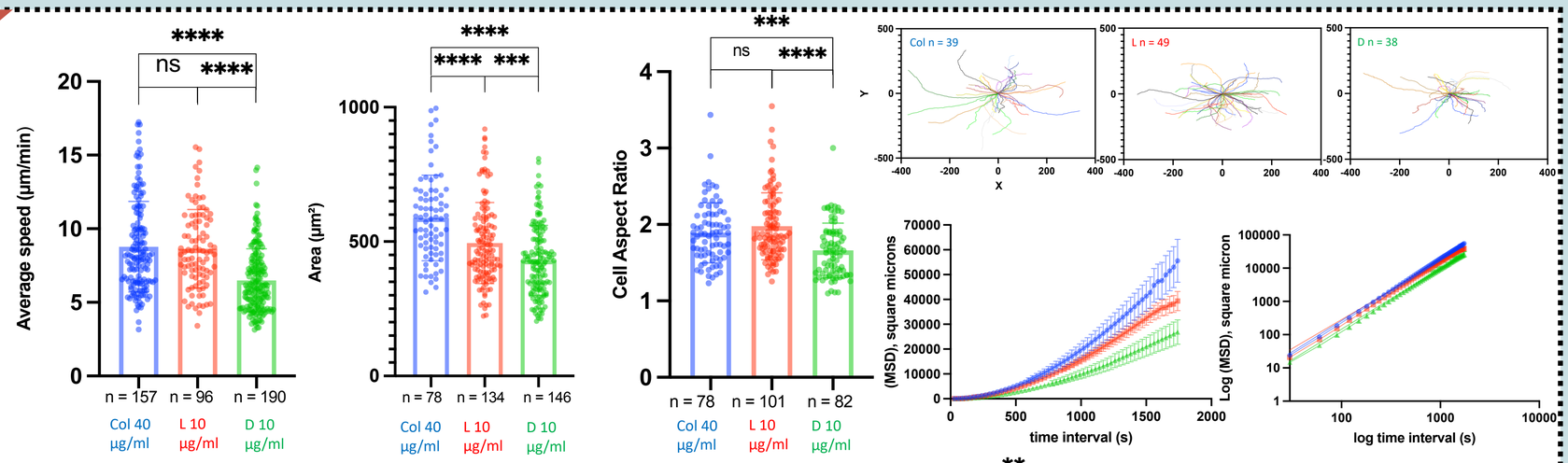
Chirality is ubiquitous in Nature, from living organisms to biomolecules, and influences fundamental processes that involve intermolecular interactions. Important biological processes are based on cell proliferation and migration, that both take place in interaction with the components of the extracellular matrix (ECM). Among them, collagen is the most abundant protein in ECM and connective tissues. Collagen consists of left-handed helical chains supercoiled into a right-handed triple helix. While various physico-chemical properties (e.g. stiffness, topography, confinement, etc.) of the cell microenvironment have been studied extensively, the influence of the ECM chirality on cellular migration has been overlooked. To address this issue, we used a microcontact printing technique to fabricate well-controlled culture surfaces coated with either collagen I as natural matrix or biomimetic matrices made of collagen-mimetic-peptides (CMPs) presenting opposite chirality (L vs. D peptides). The surfaces were characterized by circular dichroism, showing a specific polyproline type II helix (PPII) conformation of the chains. We show that D-surfaces prevent the total spreading of epithelial keratocytes which are less spread and more rounded, demonstrating that keratocytes are sensitive to the ECM chirality. Interestingly, our findings show that migrating cells on D-surfaces exhibit a lower migration speed than those on collagen I and L substrates but are significantly more persistent, suggesting that the molecular chirality of the ECM regulates key aspects of cell migration referred to as "chirotaxis". To better understand the role of the molecular chirality on cellular mechanotransduction pathways, we characterized focal adhesions and used specific inhibitors of collagen-binding integrin receptors during migration assays.

How does the matrix chirality affect cell migration ?



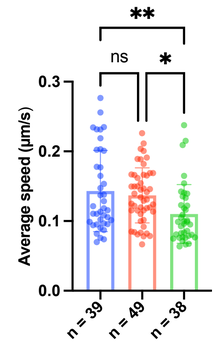
Epifluorescent images of (A) a L-dye peptide and (B) a D-dye peptide homogeneous coatings



✓ Speed: Col: 8.8 ± 3.4µm/min
L: 8.6 ± 2.7 µm/min
D: 6.7 ± 2.3 µm/min

✓ Area: Col: 588 ± 159 µm²
L: 491 ± 158 µm²
D: 409 ± 134 µm²

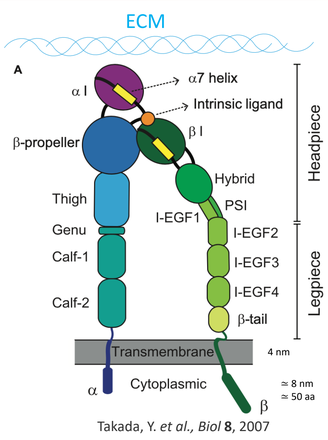
✓ CAR: Col: 1.90 ± 0.39
L: 1.98 ± 0.44
D: 1.66 ± 0.36



Slope	1.874	1.744	1.832
S (µm/s)	0.143	0.137	0.110
P (µm)	45.76	46,60	75,37
u (µm²/S)	3.3	3.2	4.2

Epithelial keratocytes are slower on D-substrates but more persistent

Cell-matrix interactions

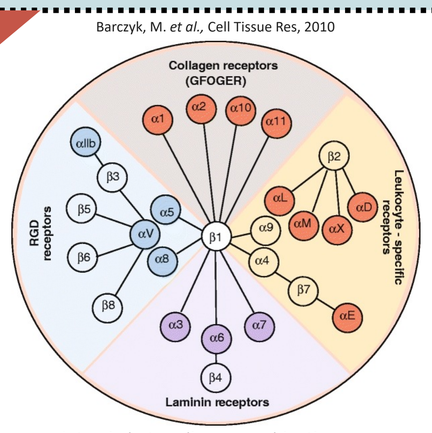


Takada, Y. *et al., Biol* **8**, 2007

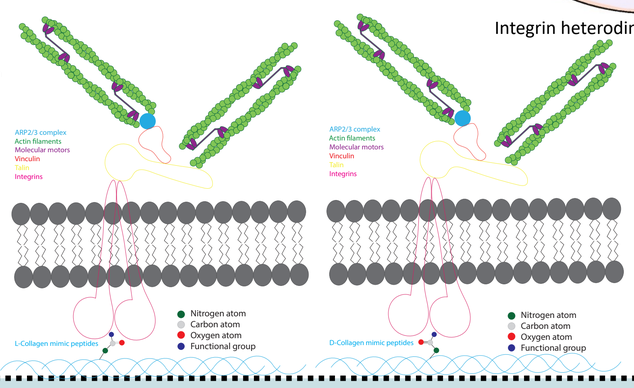
Schematic representation of the integrin heterodimer

Which integrins are sensitive to the matrix chirality ?

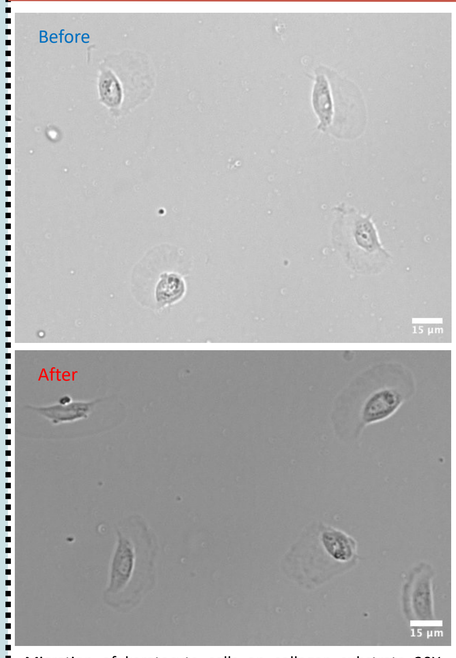
Barczyk, M. *et al.*, Cell Tissue Res, 2010



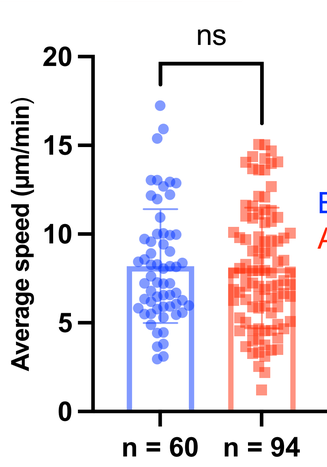
Integrin heterodimers combination



Inhibition of $\alpha_2\beta_1$



Migration of keratocyte cells on collagen substrate 20X, before (up) and after treatment (down) with $\alpha_2\beta_1$ antibody

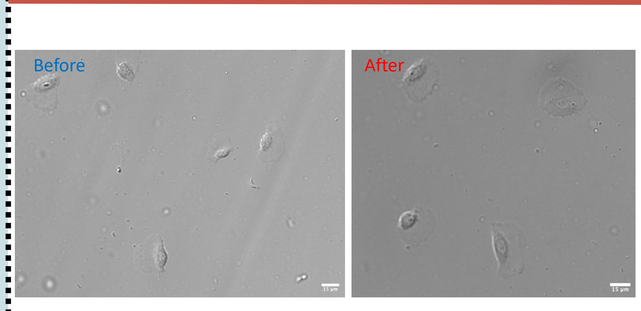


Control : 8.2 ± 3.2 µm/min
Inhibition : 8.1 ± 3.4 µm/min

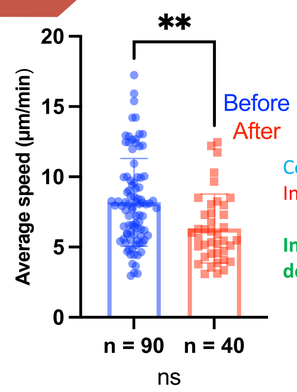
The inhibition of the collagen receptor $\alpha_2\beta_1$ does not affect the migration speed on collagen type I.

What are the integrin receptors involved in chirotaxis ?

Inhibition of $\alpha_5\beta_1$

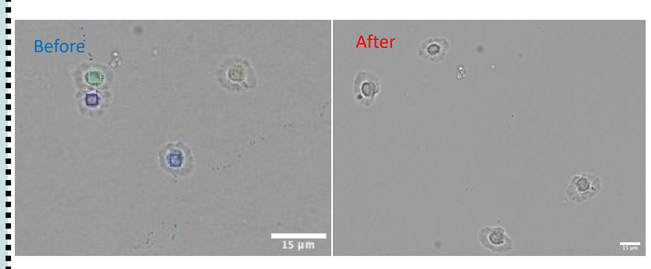


Keratocytes migration on collagen substrate 20X, before (left) and after treatment (right) with $\alpha_5\beta_1$ antibody



Control : 8.2 ± 3.1 µm/min
Inhibition : 6.3 ± 2.4 µm/min

Inhibition significantly decreases the cellular speed



Keratocytes migration on D-substrate 20X, before (left) and after treatment (right) with $\alpha_5\beta_1$ antibody

Control : 6.3 ± 1.8 µm/min
Inhibition : 6.2 ± 2.1 µm/min

No modification of cell velocity

$\alpha_5\beta_1$ is not sensitive to the matrix chirality

Conclusion and prospects

The matrix chirality modulated:

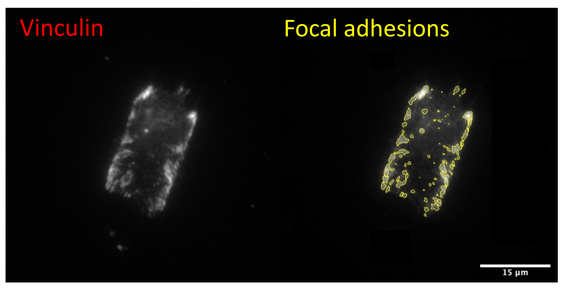
- Speed
- Spreading
- Shape
- Persistence length

→ $\alpha_2\beta_1$ integrins inhibition: no impact on speed

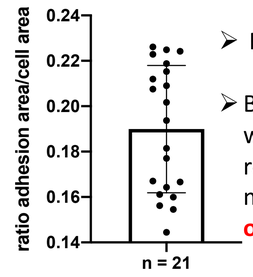
→ $\alpha_5\beta_1$ integrins inhibition: impact on collagen but no impact on D

→ $\alpha_V\beta_3$ integrins: are interesting candidates??

Could we characterize focal adhesions on L and D substrates ?



Epifluorescent images of keratocytes migrating on collagen microstripes. Scale bar: 15 µm.



- Ratio : 0.19 ± 0.03
- By immunochemistry techniques, we can visualize vinculin recruitment on collagen microstripes. **What about those on chiral substrates ?**

Acknowledgement: Alexandre Remson is financially supported by FNRS.

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