Migration of epithelial keratocyte cells on supramolecular chiral collagen-mimic peptides

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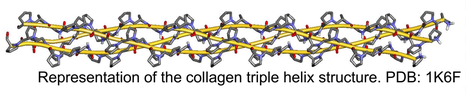
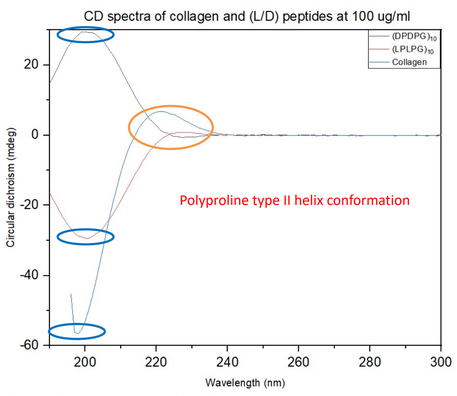
Chirality is ubiquitous in Nature, from living organisms to biomolecules, and influences fundamental processes that involve intermolecular interactions. Interestingly, many of these biological processes are based on cell proliferation and migration, that both rely on interactions with proteins of the extracellular matrix (ECM). While various physico-chemical cues of the cell microenvironment have been studied extensively, the influence of the ECM chirality on cell migration has been overlooked. To explore this issue, we propose to use multi-hierarchical self-assemblies of (oligo)peptides to design well-defined in vitro migration assays. By using this multidisciplinary approach, we will investigate the effect of chirality, from the molecular to the supramolecular level, on the migration of epithelial cells in 2D and 3D microenvironments.
We aim at understanding how molecular and supramolecular chirality can modulate integrin-based mechanotransduction mechanisms involved in cell migration

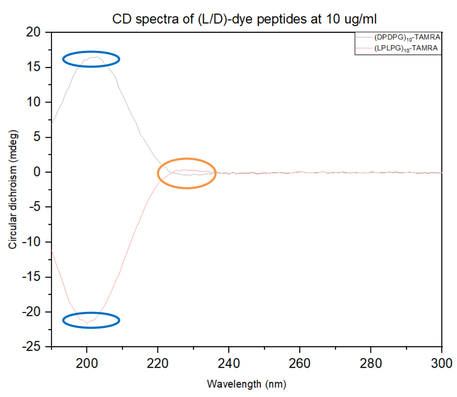
Structures of L/D and dyes peptides



Representation of the collagen triple helix structure. PDB: 1K6F

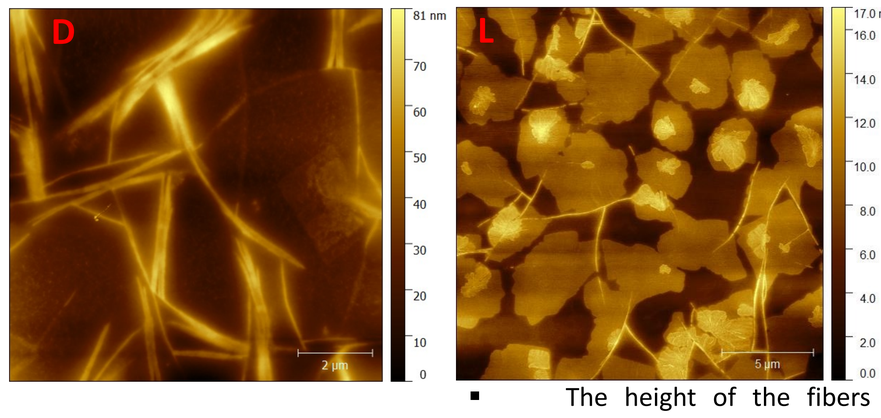
$(LPLPG)_{10}$: Collagen-mimic oligopeptides with L chirality
$(DPDPG)_{10}$: Collagen-mimic oligopeptides with D chirality
$(LPLPG)_{10}$-TAMRA: Collagen-mimic oligopeptides with TAMRA dye and L chirality
$(DPDPG)_{10}$TAMRA: Collagen-mimic oligopeptides with TAMRA dye and D chirality





✓ CD signature similar to collagen
✓ **Positive/negative peak** (L/D) at 225 nm and **negative/positive peak** (L/D) at 200 nm
✓ Self-assembly of the chains in triple helix with PPII conformation

Morphological analysis (AFM)

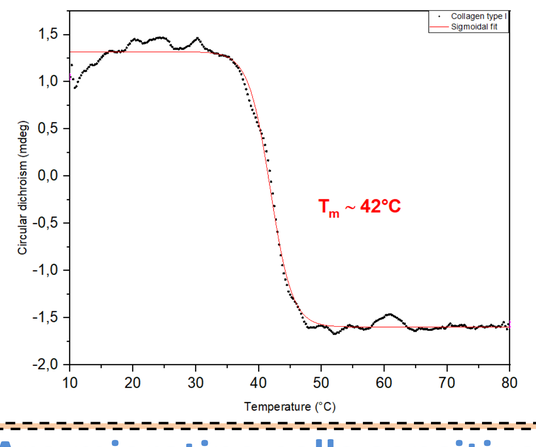


	D peptide	L peptide
Heigth (nm)	15.9 ± 3.1	7.3 ± 1.3
Width (nm)	115 ± 11	132 ± 15
Length (µm)	0.5-6.5	0.5-4.6

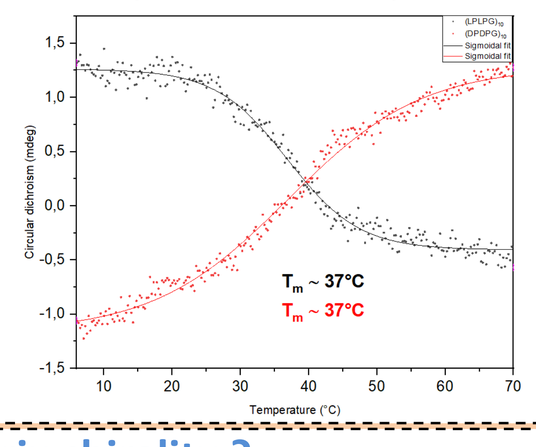
- The height of the fibers seems to increase when the concentration is quadrupled
- At lowest concentration, fiber formation seems to require a first layer of matter

Thermal melting experiments of chiral peptides

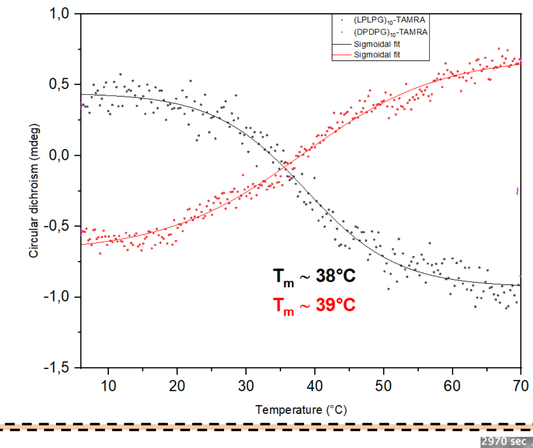
Collagen at 100 ug/ml



$(LPLPG)_{10}$ 100 ug/ml & $(DPDPG)_{10}$ 100 ug/ml

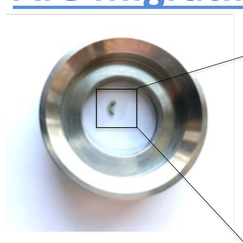


$(LPLPG)_{10}$-TAMRA 10 ug/ml & $DPDG)_{10}$-TAMRA 10 ug/ml



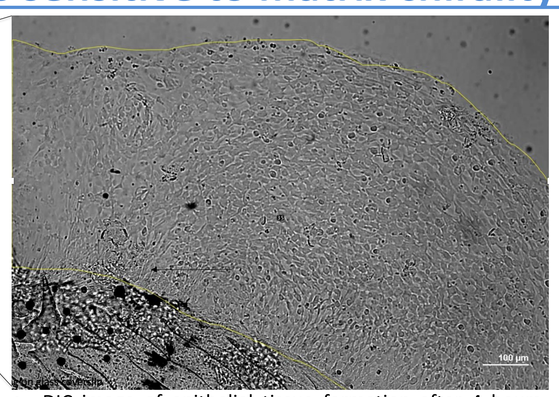
- Highest melting temperature for collagen type I
- Sigmoidal curve more marked for collagen sample
- Slow increase in melting temperature for (L/D)—dye peptides

Are migrating cells sensitive to matrix chirality ?

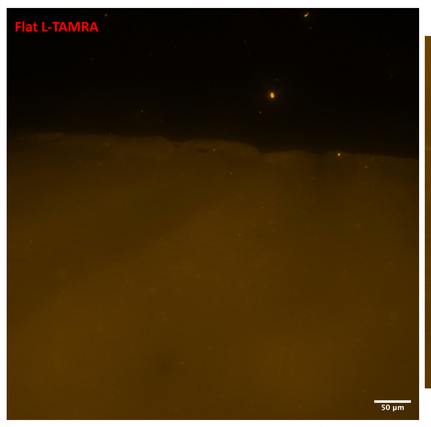


- A fish scale is deposited on a glass coverslip overnight, leading to the growth of an epithelial tissue.

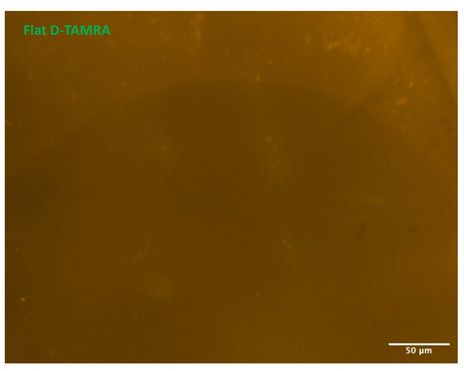
✓ Microcontact printing allows to create patern with micrometric resolution



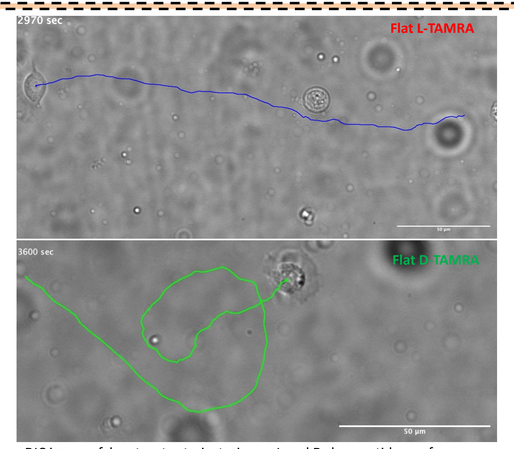
- DIC image of epithelial tissue formation after 4 hours deposition on glass coverslip



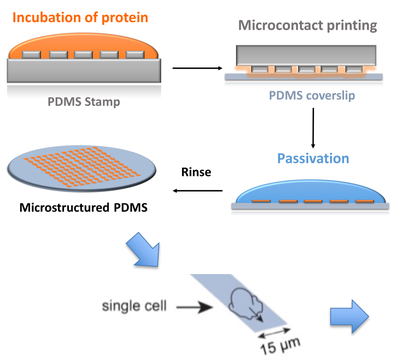
Epifluorescent image of the coverslip extremity of L-dye peptide microprinting



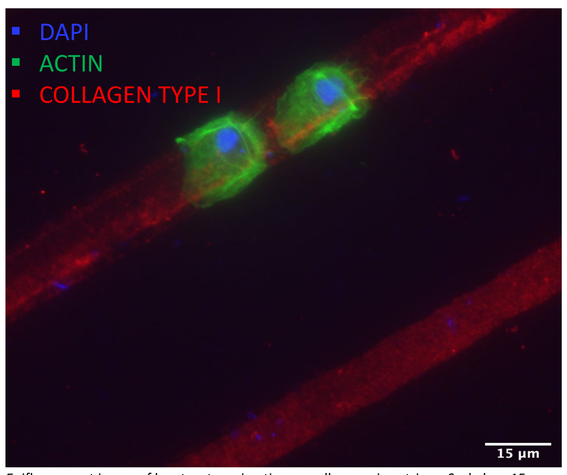
Epifluorescent image of the coverslip center of D-dye peptide microprinting



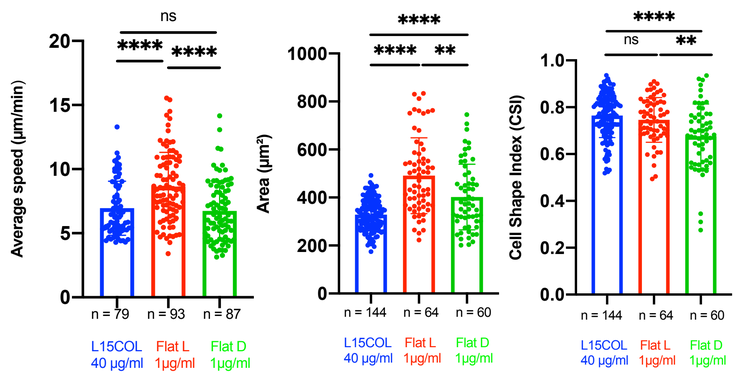
DIC image of keratocytes trajectories on L and D-dye peptides surfaces



Schematic view of a cell on microstripe



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



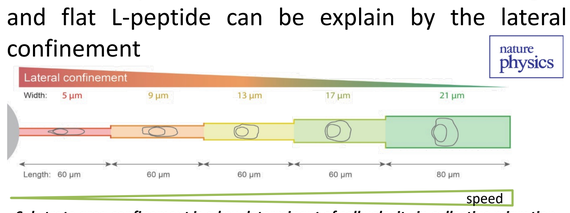
✓ Speed: COL: 7.0 ± 2.0 µm/min
L: 8.6 ± 2.7 µm/min
D: 6.7 ± 2.3 µm/min

✓ Area: COL: 330 ± 67 µm²
L: 491 ± 158 µm²
D: 402 ± 137 µm²

✓ CSI: COL: 0.77 ± 0.09
L: 0.75 ± 0.09
D: 0.68 ± 0.14

$$CSI = \frac{4\pi A}{P^2}$$

➢ The cellular parameters difference between collagen and flat L-peptide can be explain by the lateral confinement



Substrate area confinement is a key determinant of cell velocity in collective migration.
D. Mohammed *et al.* (2019)

➢ Keratocytes seem to more interact with the L-chiral substrates

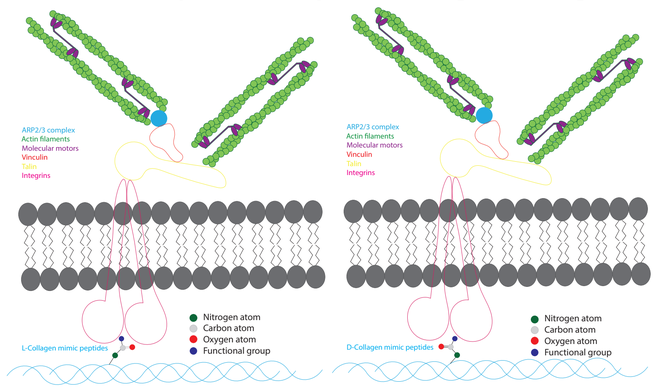
➢ Keratocyte cells are more spread when migrating on L-substrates

➢ What about on chiral L/D L15 surfaces ?

Conclusions

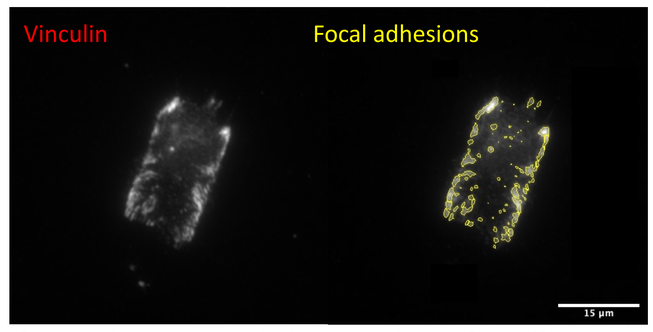
- ➢ Peptides and dyes-peptides mimic the collagen structure by conservating the polyproline type II helix conformation
- ➢ Collagen sequence leads to higher cooperativity than peptides and dyes
- ➢ AFM experiments showed that peptides mainly formed fibers
- ➢ Specific interactions between collagen and cells via integrin recruitments
- ➢ The speed of keratocytes is higher when migration occurs on the L-chiral substrate. In addition, the cellular parameters seem to indicate a better cellular behavior on this type of matrix.

Taken together, these results point the way towards original strategies to study the cell migration with enantiospecific surfaces



What's happened during the establishment of cell-matrix-interactions ?

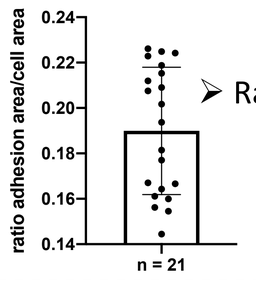
Prospect: investigating cell-matrix interactions ?



Berginski M.E., *et al.* F100 research (2013)

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

By immunochemistry techniques, we visualized vinculin recruitment on collagen microstrips. **What about those on chiral substrates ?**



➢ Ratio : 0.19 ± 0.03

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