How local changes of matrix curvature can direct collective cell migration through modulation of Erk signaling waves

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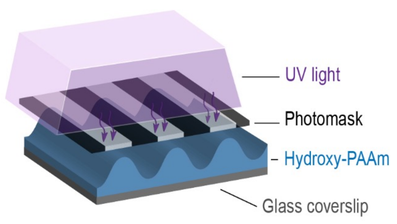
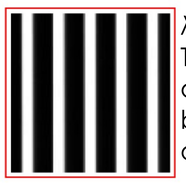
Collective migration is a key function of many epithelial tissues, both in physiological (wound healing) and pathological (cancerous metastases) processes. Recent evidence suggests that propagation waves of extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase activation determine the direction of the collective cell migration. Interestingly, accumulative evidence shows that single cells respond to cell-scale curvature variations (curvotaxis). However, it remains elusive how local changes of curvature can modulate the propagation of ERK and be integrated to coordinate the collective movement. Here we use a photopolymerization method to form in soft hydrogels well-defined corrugation patterns of different wavelengths, as observed in many native epithelial tissues. Our results show that corrugations induce a uniaxial collective flow of MDCK cells in the direction of the corrugation axis, demonstrating a curvotaxis effect on collective migration. By combining Förster resonance energy transfer(FRET)-based biosensors in MDCK cells with long time-lapse experiments, our findings show that ERK protein activation spreads from cell to cell in a defined dynamic pattern (waves) during collective cell migration on flat hydrogels. We then investigate how the modulation of the local curvature can lead to a mechanical stretch at the single cell level, which can activate ERK through epidermal growth factor receptor (EGFR) activation, and ERK activation triggers cell contraction. The contraction of the activated cell pulls neighboring cells, evoking another round of ERK activation and contraction in the neighbors. Our study raises the question of the critical role of cellular response to external stimuli such as matrix curvature in intercellular signal transduction.

Static state on corrugated hydrogels

METHOD :
Schematic representation of the UV photo-polymerization of hydroxy-PAAm hydrogels through an optical photomask



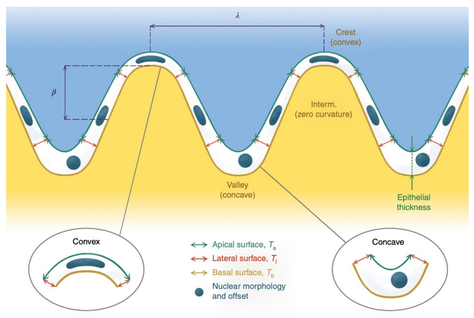
Photomask : **λ = 30 µm**



λ 30 µm :
Transparent stripes of 10 µm wide and black stripes of 20 µm wide

Luciano M. *et al*, 2021

Schematic of an epithelial monolayer grown on a corrugated polyacrylamyde hydrogel



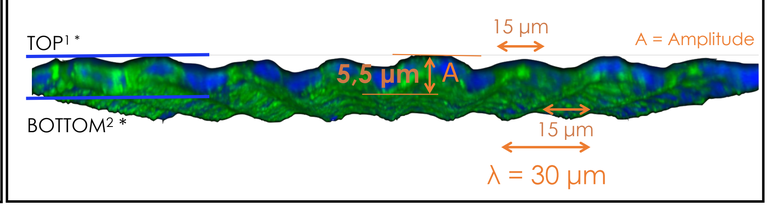
Amplitude β
Wavelength λ

Luciano M. *et al*, 2021

Polyacrylamide hydrogel : characteristics

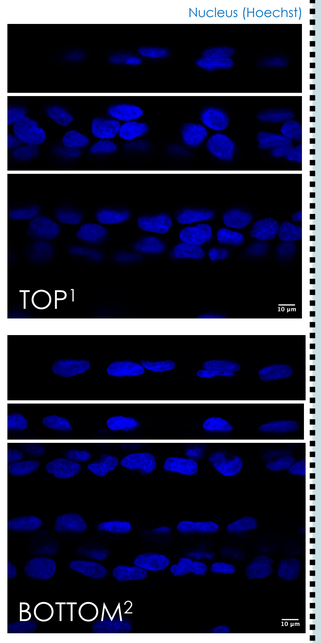
Nucleus (Hoechst)
Actin (AlexaFluor 488)

CORRUGATED (λ = 30 µm) -> SYMMETRICAL



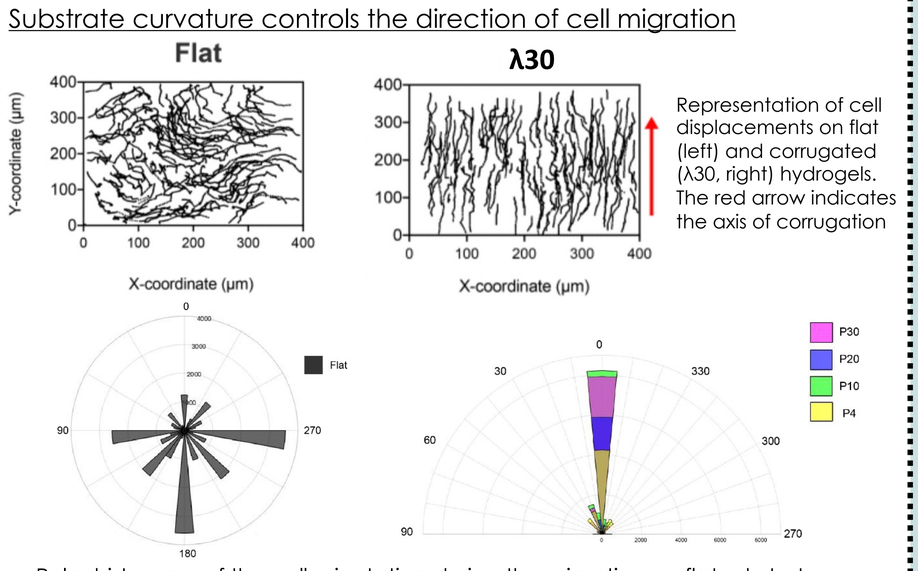
*Nuclei align according to the curvature of the substrate

Nucleus (Hoechst)



Dynamic state on corrugated hydrogels

Substrate curvature controls the direction of cell migration

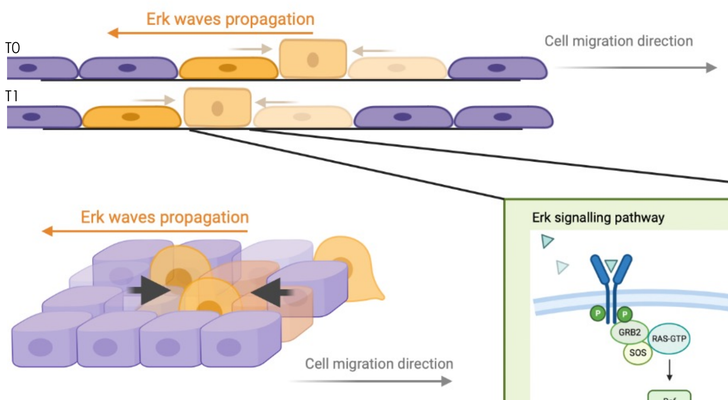


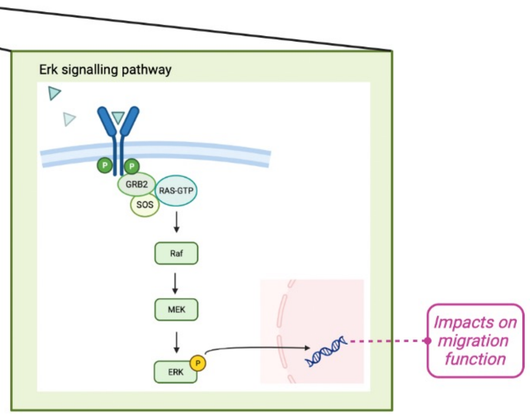
Representation of cell displacements on flat (left) and corrugated (λ30, right) hydrogels. The red arrow indicates the axis of corrugation

- Polar histogram of the cell orientation during the migration on flat substrates showing the random orientation of cell migration (left)
- Polar histogram of the cell orientation during the migration on P4, P10, P20 and P30 substrates (right)

Luciano M. *et al*, 2021

Erk waves are activated by mechanical forces transmitted via cell-cell junctions

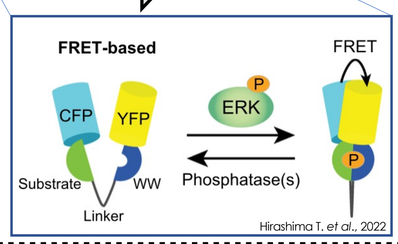




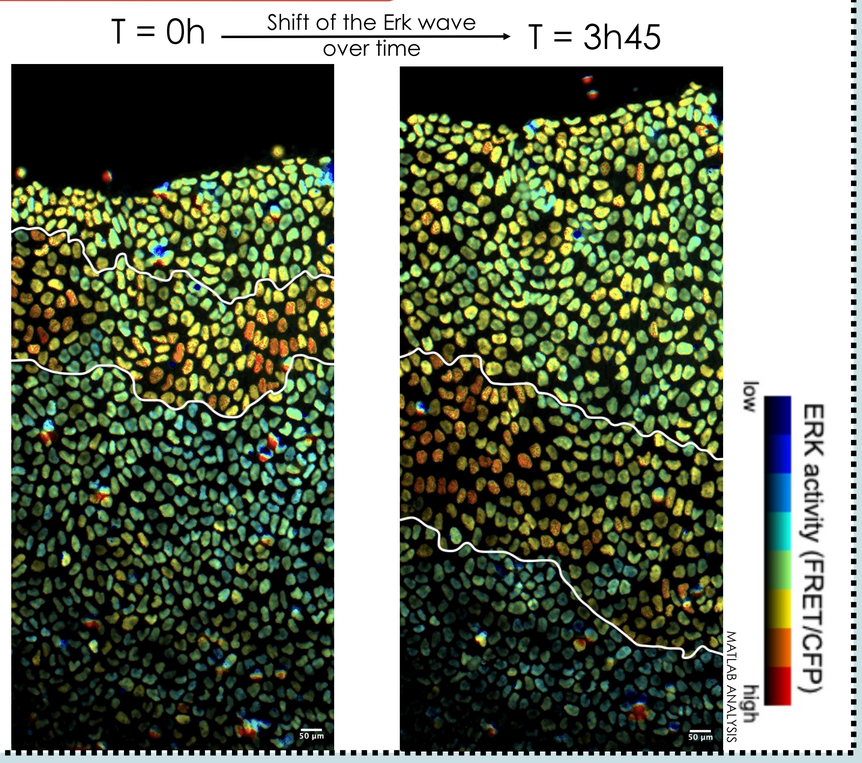
- Erk waves propagation moves from the leader to the follower cells due to the contraction-extension of the cell
- Erk waves direction moves in the opposite way of cell migration

Adapted from Hirashima T. *et al.*, 2022

Time lapse: visualization of epithelial cells (MDCK) migration on flat hydrogels expressing the ERK probe

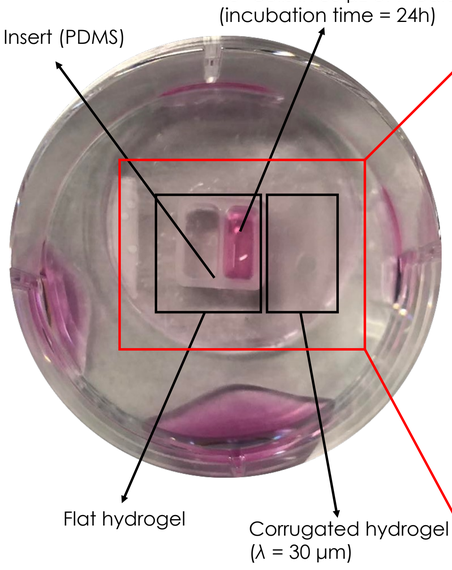


Hirashima T. *et al.*, 2022



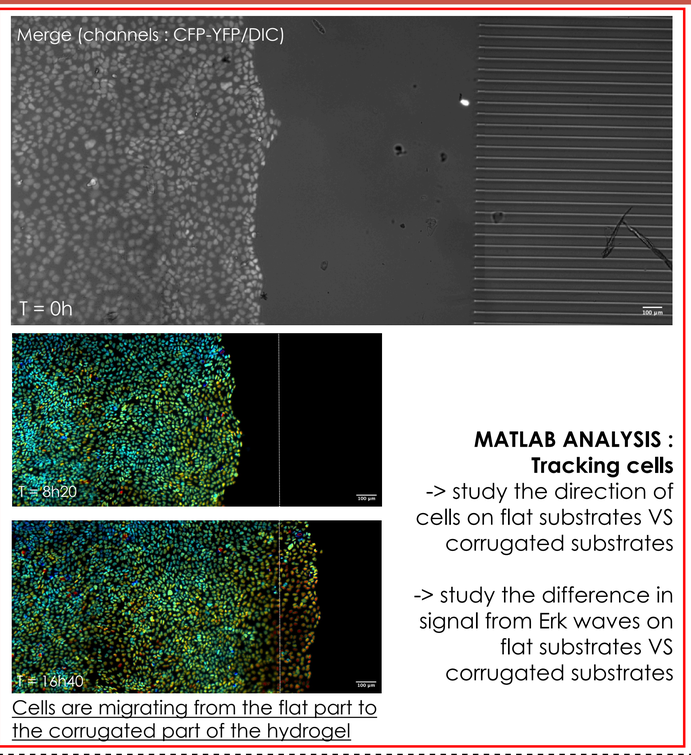
ERK analysis : migration of MDCK cells from a flat to a corrugated hydrogel

METHOD :



Culturing MDCK cells expressing the ERK probe in a PDMS insert glued to the flat part of the hydrogel (flat/P30)

DYNAMIC ANALYSIS

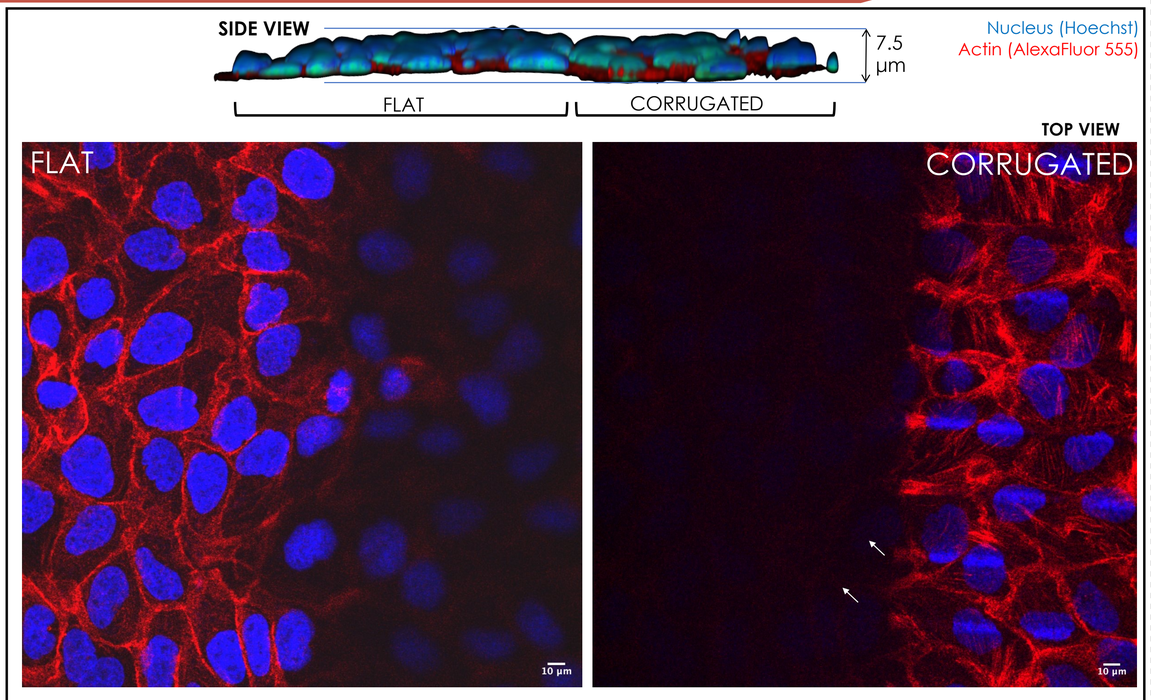


Cells are migrating from the flat part to the corrugated part of the hydrogel

MATLAB ANALYSIS :
Tracking cells
-> study the direction of cells on flat substrates VS corrugated substrates

-> study the difference in signal from Erk waves on flat substrates VS corrugated substrates

STATIC ANALYSIS



Actin filaments and nuclei adapt to the concave and convex zones valleys of the hydrogels

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INFLUX

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