OXYGEN GRADIENT GENERATORS INCLUDING OXYGEN SENSORS TO INVESTIGATE CELL AEROTAXIS

LABORATORY : Institut Lumière Matière

LEVEL : M1
TEAM(S) : BIOPHYSIQUE

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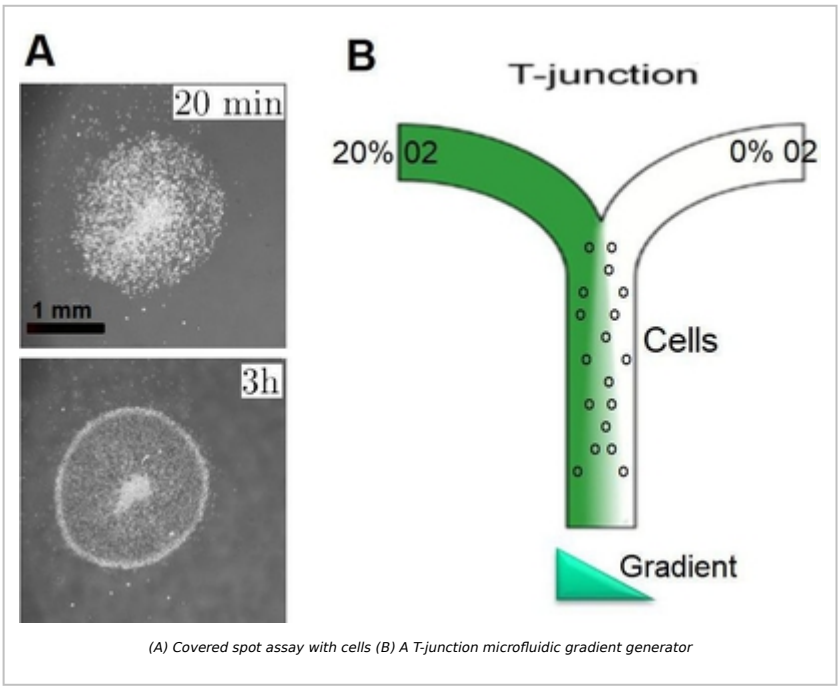
KEYWORD(S) : Microfabrication / Fluorescence Microscopy / Cell tracking

SCIENTIFIC CONTEXT :



(A) Covered spot assay with cells (B) A T-junction microfluidic gradient generator

It has been known for the last three decades that cells are able to detect and adapt to various concentrations of oxygen (O2) by gene regulation as just highlighted by the Nobel Prize in Medicine 2019 [1]. It is also well known that bacteria, rather than regulating genes, move toward O2, a mechanism called aerotaxis [2]. Epithelial cells also exhibit directed migration toward oxygen: after covering an epithelial cell monolayer by a coverglass non permeable to O2, peripheral cells exhibit a strong outward directional migration to escape hypoxia from the center of the colony [3]. Following that spot assay, we showed at iLM that the social amoeba Dictyostelium displays a spectacular phenotype when cells consumed their O2: most cells move quickly outward of the hypoxia area, forming a dense expending ring moving at constant speed [4]. This clear aerotactic collective response induced by self-generated O2 gradient was further investigated using a two-layer microfluidic device with two upper gas channels controlling the O2 gradient inside a cell media channels with single cells at low density. However, the molecular nature of this O2 directed migration remains elusive as well as the exact sensing mechanism: do cells respond to temporal changes when they move in the gradient as bacteria or do they feel at any time the spatial gradient by sensing a different oxygen concentration at their front and at their tail ?

MISSIONS :

To answer these questions we need to vary the imposed gradient conditions and be able to change them more dynamically than with the two-layer system. A simple microfluidic T-junction between an oxygenated and a degassed media (green and white channels in the Fig. 1F) arriving in a region where cells are plated presents a well-defined and measurable gradient region. We plan to control both spatial and temporal gradients with microfluidics by just changing temporally the flowrate in the two input channels. Cell trajectories will be followed by videomicroscopy and very importantly, O_2 gradient will be measured will be measured using a bottom glass surface covered by an oxygen sensing film based on the phosphorescence quenching of porphyrins molecules embedded in a thin PDMS layer.

Tasks during the internship.

- Prepare sensing film and calibrate their sensitivity (Month 1)

- Prepare T-junctions and test them with the sensing film (Monthes 1 and 2)

- Do a test experiment with cells inside the T-junction (Month 2)

OUTLOOKS :

We expect to understand better aerotaxis.

BIBLIOGRAPHY :

[1] https://www.nobelprize.org/prizes/medicine/2019/press-release/ NobelPrize.org.

[2] Micha Alder et al. Studies of bacterial aerotaxis in a microfluidic device. Lab Chip, 12(22) :4835_4847, November 2012.

[3] M. Deygas et al.. Redox regulation of EGFR steers migration of hypoxic mammary cells towards oxygen. To appear in Nat. Comm. (2018).

[4] O. Cochet-Escartin et al., “Hypoxia triggers collective aerotactic migration in Dictyostelium discoideum, eLife (2021),”