



**HAL**  
open science

## How hydrodynamics and species transport influence the 3D microenvironment of cells seeded in hydrogel scaffolds

Martial Bankoué Ntaté, Jérôme Grenier, Soukaina El Hajj, Magali Dupuy, Cyril Breton, Rachida Aid, Clément Journée, Robin Siadous, Jerome Ligneron, Joëlle Amédée, et al.

### ► To cite this version:

Martial Bankoué Ntaté, Jérôme Grenier, Soukaina El Hajj, Magali Dupuy, Cyril Breton, et al.. How hydrodynamics and species transport influence the 3D microenvironment of cells seeded in hydrogel scaffolds. ESB 2022 32nd Annual Conference of the European Society for Biomaterials, Sep 2022, Bordeaux, France. . hal-03845219

**HAL Id: hal-03845219**

**<https://hal.science/hal-03845219>**

Submitted on 6 Feb 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# How hydrodynamics and species transport influence the 3D microenvironment of cells seeded in hydrogel scaffolds

Martial Bankoué Ntaté<sup>1\*</sup>, Jérôme Grenier<sup>1</sup>, Soukaina El Hajj<sup>2</sup>, Magali Dupuy<sup>1</sup>, Cyril Breton<sup>1</sup>, Rachida Aid<sup>3</sup>, Clément Journé<sup>3</sup>, Robin Siadous<sup>4</sup>, Jérôme Ligneron<sup>4</sup>, Joelle Amédée<sup>4</sup>, Didier Letourneur<sup>3</sup>, Bertrand David<sup>2</sup>, Hervé Duval<sup>1</sup>

<sup>1</sup>LGPM, CentraleSupélec, Université Paris-Saclay, Gif-sur-Yvette, France

<sup>2</sup>LMPS, UMR CNRS 9026, CentraleSupélec, Université Paris-Saclay, Gif-sur-Yvette, France

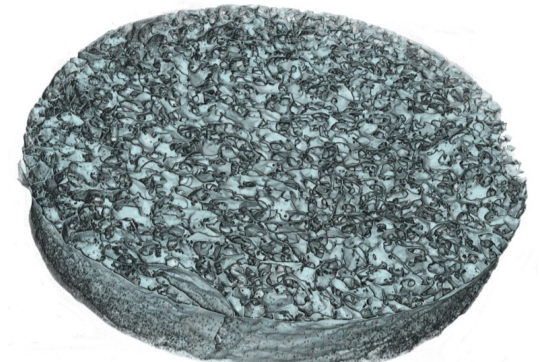

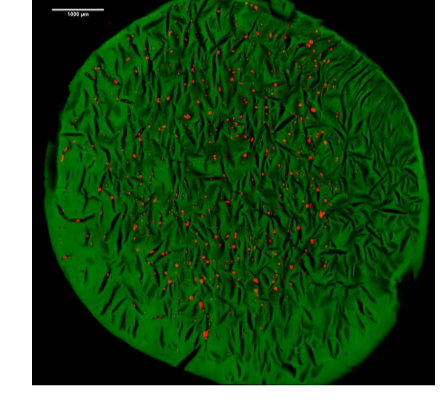

<sup>3</sup>LVTS, Inserm U1148, Université Paris Cité, Université Sorbonne Paris Nord, Paris, France

<sup>4</sup>Laboratoire BIOTIS, Inserm U1026, Université de Bordeaux, Bordeaux, France

\*martial.bankoue-ntate@centralesupelec.fr



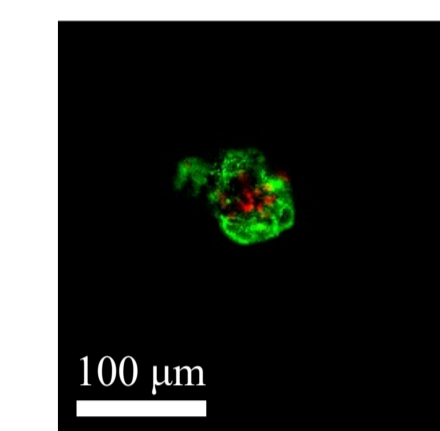
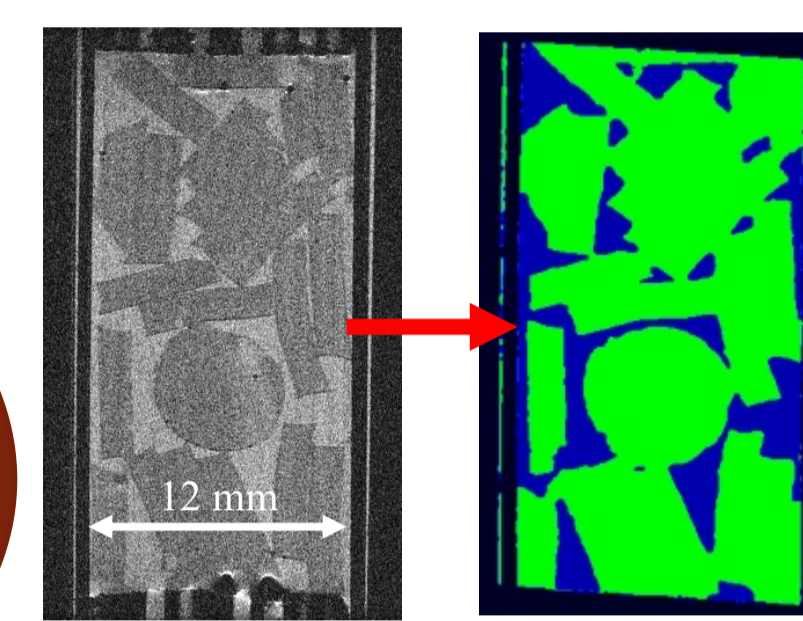
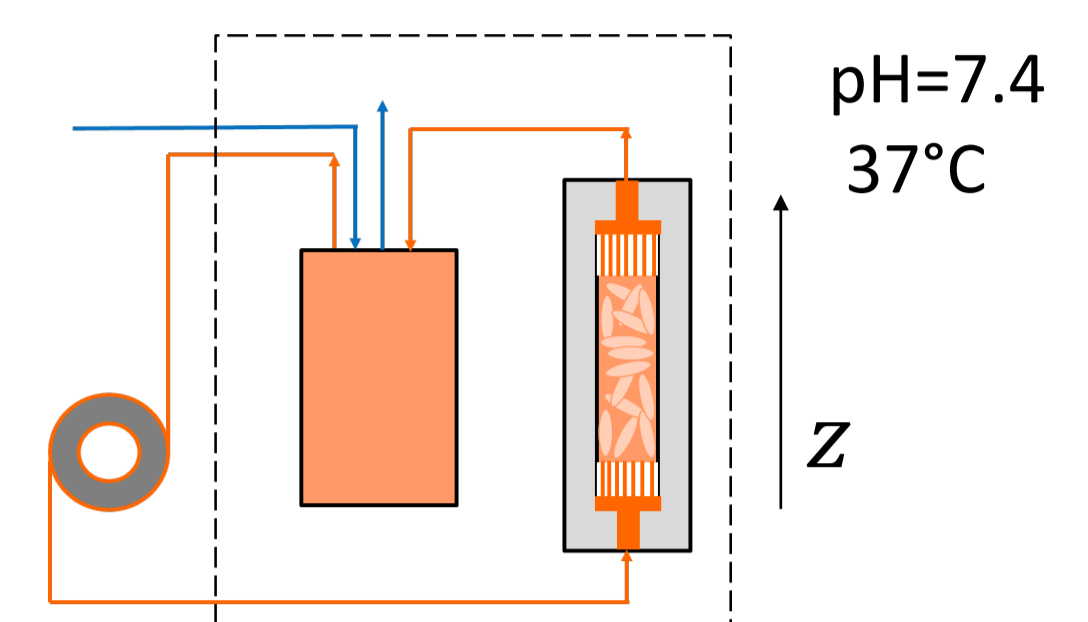
## Context

- 



- Bone tissue engineering
    - In vitro model of bone reconstruction
  - Porous scaffolds seeded with MSCs cultured in a perfusion bioreactor<sup>1</sup>
    - Pullulan/dextran porous hydrogel scaffolds<sup>2,3</sup> → 3D microenvironment
    - Spontaneous formation of spheroids<sup>4</sup>
  - Perfusion flow
    - long-term delivery of oxygen and nutrients → **viability**
    - mechanical stimuli → **cell fate** (via mechano-transduction)
  - Preliminary tests<sup>5</sup> with MC3T3E1 mouse preosteoblast cells

Aim: Develop a bioreactor digital twin to determine the chemical/mechanical microenvironment seen by the cells

## Custom-made perfusion bioreactor

- MC3T3E1 mouse preosteoblasts
  - Scaffolds: disks of 10 mm diameter and 2 mm thickness
  - Initial cell seeding: 400,000 per scaffold
  - Fixed bed: 48 scaffolds randomly stacked
  - Culture medium:  $\alpha$ -MEM, supplemented by antibiotics, equilibrated at pH=7.4 by CO<sub>2</sub>-bubbling
  - Perfusion rate: 10 mL.min<sup>-1</sup>
  - 1 to 3 weeks of dynamic culture
- 3D geometry of the stack
    - 7T MRI, resolution: 55  $\mu$ m
    - Image processing using Avizo
  - Cell number and viability during dynamic culture
    - CLSM → stable number of 100,000 cells per scaffold, spheroid mean diameter of 75  $\mu$ m
    - Live/Dead assay → viability of 80%



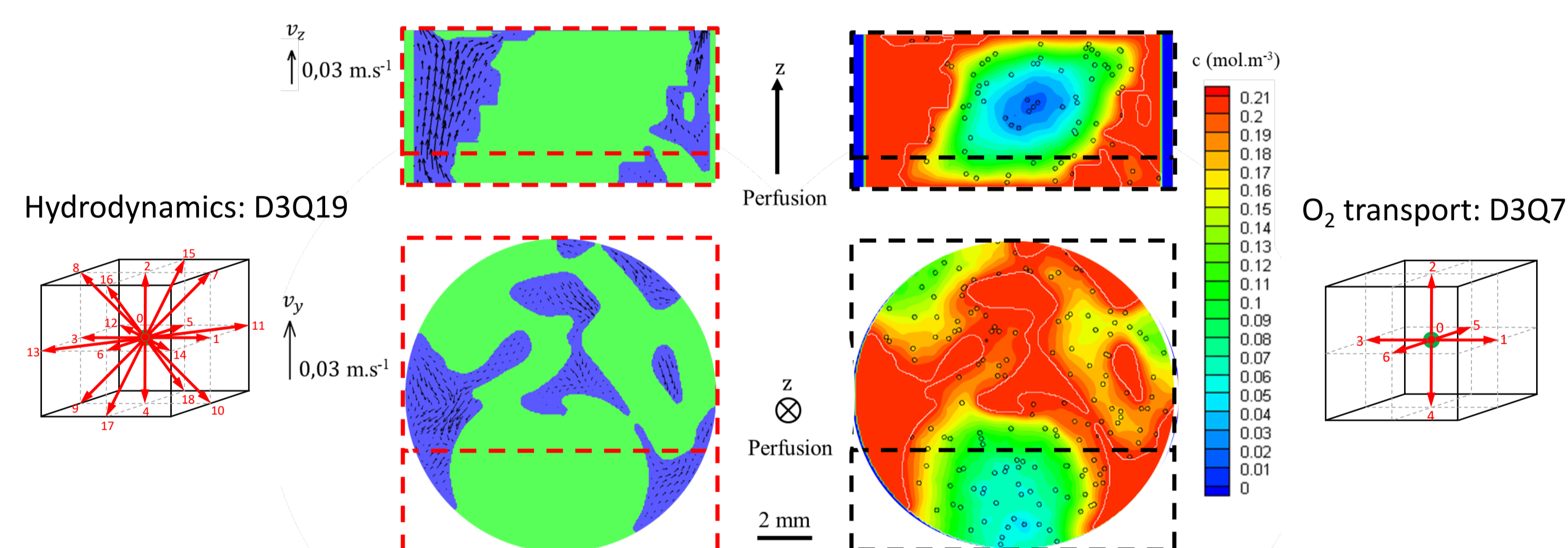
Green: viable cells  
Red: dead cells

## Bioreactor modeling

- (1)  $\vec{\nabla} \cdot \vec{v} = 0$
- (2)  $\rho \frac{\partial v_\alpha}{\partial t} + \rho \vec{v} \cdot \vec{\nabla} v_\alpha = \rho g_\alpha - \frac{\partial p}{\partial x_\alpha} + \mu \Delta v_\alpha \quad \alpha = x, y, z$  Hydrodynamics
- (3)  $\frac{\partial C_{O_2}}{\partial t} + \vec{v} \cdot (C_{O_2} \vec{v}) = -D_{O_2} \Delta C_{O_2} + \Omega_{O_2}$  Species (O<sub>2</sub>) transport
- $$\Omega_{O_2} = \begin{cases} \frac{V_{max}}{\vartheta} \frac{C_{O_2}}{C_{O_2} + K_M} & \rightarrow \text{O}_2 \text{ consumption by spheroids} \\ 0 & \rightarrow \text{anywhere else} \end{cases}$$
- $\vec{v}$ : Fluid velocity (m.s<sup>-1</sup>)
  - $C_{O_2}$ : O<sub>2</sub> concentration (mol.m<sup>-3</sup>)
  - $D_{O_2}$ : O<sub>2</sub> diffusion coefficient (3 10<sup>-9</sup> m<sup>2</sup>.s<sup>-1</sup> in the fluid, 1.6 10<sup>-9</sup> m<sup>2</sup>.s<sup>-1</sup> in the hydrogel)
  - $\vartheta$ : cell volume (3000  $\mu$ m<sup>3</sup>)
  - $V_{max}$ : maximum O<sub>2</sub> consumption rate (4 10<sup>-17</sup> mol.cell<sup>-1</sup>.s<sup>-1</sup>)
  - $K_M$ : Michaelis-Menten constant (6 10<sup>-3</sup> mol.m<sup>-3</sup>)

## Hydrodynamics and oxygen transport

- Scaffolds virtually seeded with 135  $\mu$ m-diameter spheroids
- Each spheroid represented by eight 55  $\mu$ m-voxels
- Number of spheroids adjusted to obtain about 100,000 cells per scaffolds



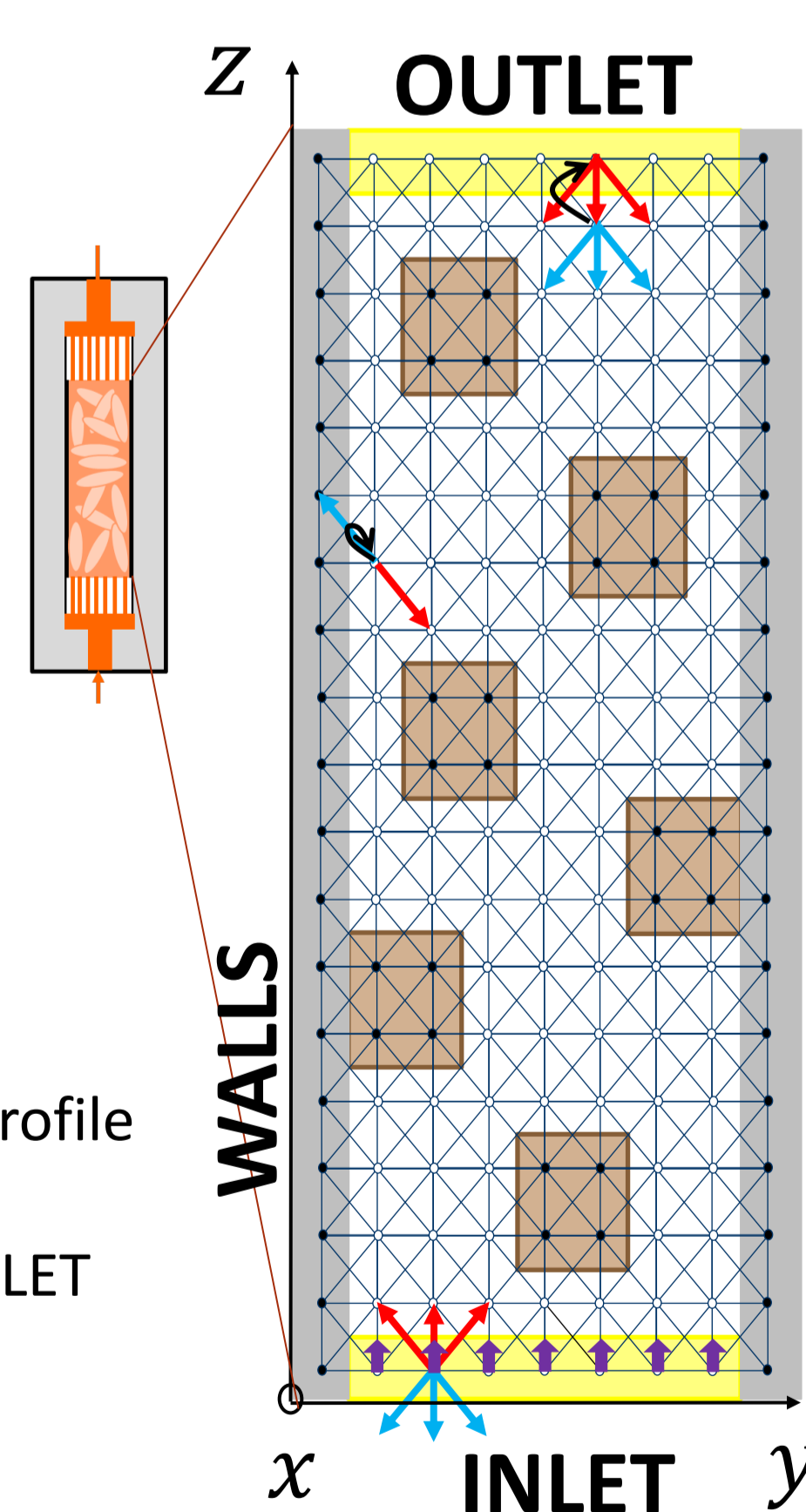
- Maximum fluid interstitial velocity reaches **27 mm.s<sup>-1</sup>**
- Wall shear stress from **0.1 mPa to 100 mPa**, average value of **25 mPa**
- **98%** of the total hydrogel volume is **non hypoxic** (criterion:  $C_{O_2} > K_M$ )
- Consistent with the viability assessment of the cells

## LBM scheme and boundary conditions

- Simulation of hydrodynamics and O<sub>2</sub> transport using **Lattice Boltzmann Methods (LBMs)**
  - Easily handle complex geometries such as porous media
  - Use « directly » the 3D images acquired by  $\mu$ CT<sup>6</sup>, MRI, or OCT

### LBM principle

- 3D space mapped on a regular lattice
- Fluid (resp. species) represented by a population of fictitious particles with a discrete and finite velocity distribution ( $Q$  particle velocity vectors)
- Particles constrained to move along the lattice
- At each time step, dynamics divided into two stages
  - Propagation of the particles from node to node
  - Collisions between particles located at the same node
- Pressure  $p$ , hydrodynamic velocity  $\vec{v}$ , (resp. O<sub>2</sub> concentration  $C_{O_2}$ ) obtained by taking appropriate moments of the velocity distribution function



### Boundary conditions

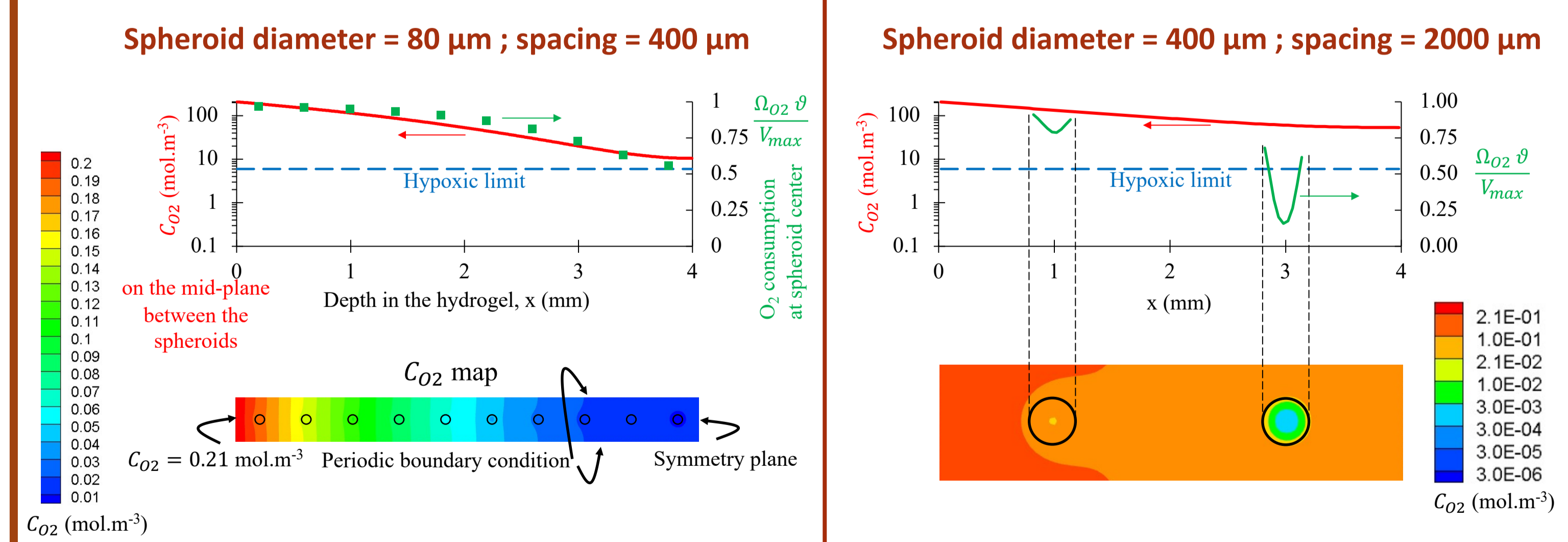
- Flat and constant fluid velocity (resp. O<sub>2</sub> concentration) profile at the **INLET**
- Zero velocity (resp. O<sub>2</sub> concentration) gradient at the **OUTLET**
- No-slip condition (resp. zero-flux) at impermeable **WALLS**: bounce-back rule

### Characteristics

- Two-relaxation-time collision operators (TRT)<sup>7,8</sup>
- $Q = 19$  particle velocities for the hydrodynamics → D3Q19
- $Q = 7$  particle velocities for the species transport → D3Q7

## At the scaffold scale

- Simulation of O<sub>2</sub> transport with a higher spatial resolution (10  $\mu$ m)
- Model configuration: spheroids of same diameter evenly spaced in a hydrogel scaffold
- 100,000 cells per scaffold



→ no hypoxia up to 4 mm depth

→ spheroid at x = 3 mm has a hypoxic core

Oxygen state depends on cell arrangement within the scaffold

## Acknowledgements

This work is supported by the "IDI 2016" project funded by the IDEX Paris-Saclay (ANR-11-IDEX-0003-02), by the LabEx LaSIPS (ANR-10-LABX-0032-LaSIPS), and by the "HydroOs" project (ANR-21-CE18-0010-01).

## References

- David, B. *et al.*, 2011. Tissue Eng. Part C Methods. 17, 505–516.
- Fricain, J.C. *et al.*, 2013. Biomaterials. 34, 2947–2959.
- Grenier, J. *et al.*, 2019. Acta Biomater. 94, 195–203.
- Guerrero, J. *et al.*, 2018. J. Tissue Eng. Regen. Med. 12, e1936-e1949.
- Grenier J., PhD Thesis, Paris-Saclay University, 2019.
- Thibeaux, R. *et al.*, 2019. Biotechnol. Prog. 35, e2880, 13 pages.
- d'Humières, D., Ginzburg, I., 2003. Physical Review E 68, 066614.
- Ginzburg, I., 2017. Physical Review E 95, 013304.

## Conclusion & perspectives

- Present approach well suited to predict both (i) fluid flow in the macroporosity of the scaffold stack, (ii) the oxygen state experienced by the cells within the scaffolds

### Next:

- Co-culture of human umbilical vein endothelial cells and human mesenchymal stem cells
- Optical coherence tomography (OCT) to get a better spatial resolution and capture the microporosity of the porous hydrogel scaffolds