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

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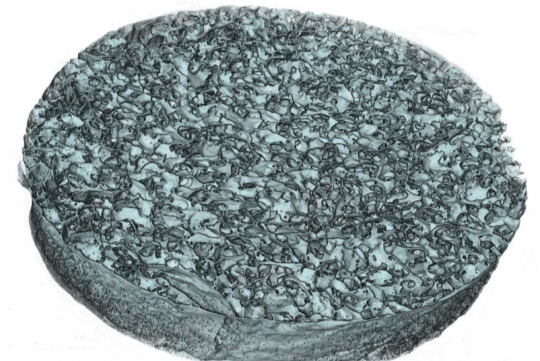
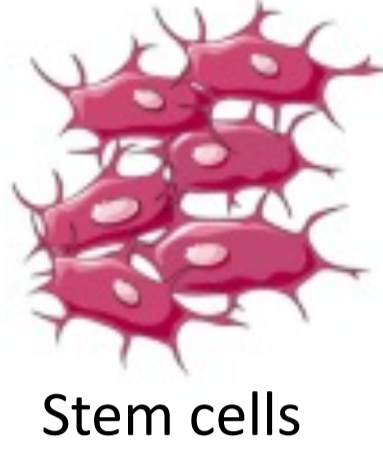
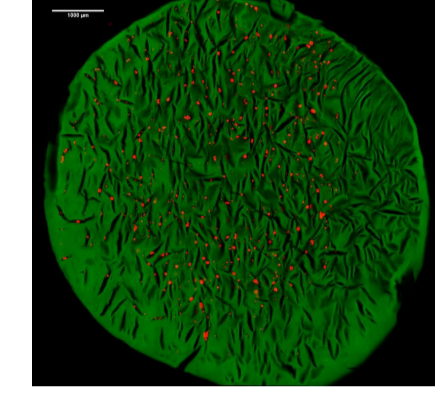

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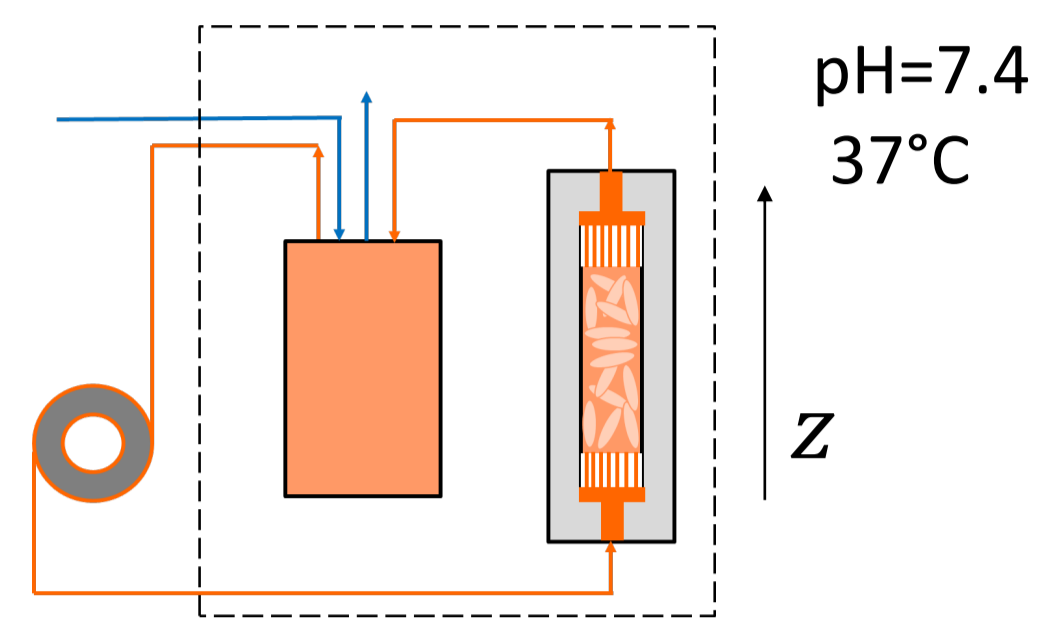


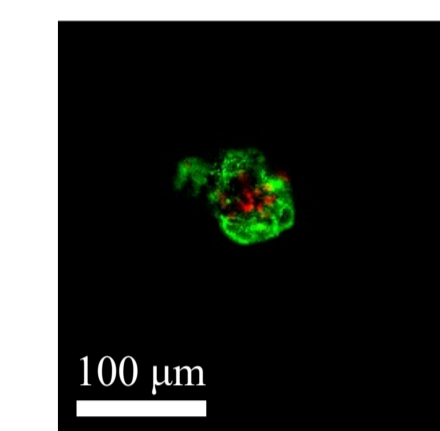
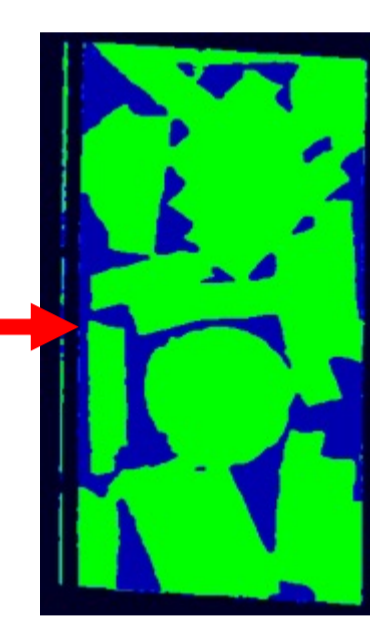
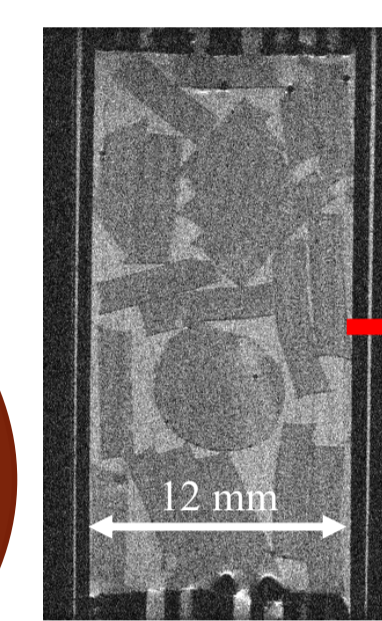
Context

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- Bone tissue engineering
 - In vitro model of bone reconstruction
 - Porous scaffolds seeded with MSCs cultured in a perfusion bioreactor¹
 - Pullulan/dextran porous hydrogel scaffolds^{2,3} → 3D microenvironment
 - Spontaneous formation of spheroids⁴
 - Perfusion flow
 - long-term delivery of oxygen and nutrients → **viability**
 - mechanical stimuli → **cell fate** (via mechano-transduction)
 - Preliminary tests⁵ 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: α -MEM, supplemented by antibiotics, equilibrated at pH=7.4 by CO₂-bubbling
 - Perfusion rate: 10 mL.min⁻¹
 - 1 to 3 weeks of dynamic culture
- 
- 3D geometry of the stack
 - 7T MRI, resolution: 55 μ 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 μ m
 - Live/Dead assay → viability of 80%



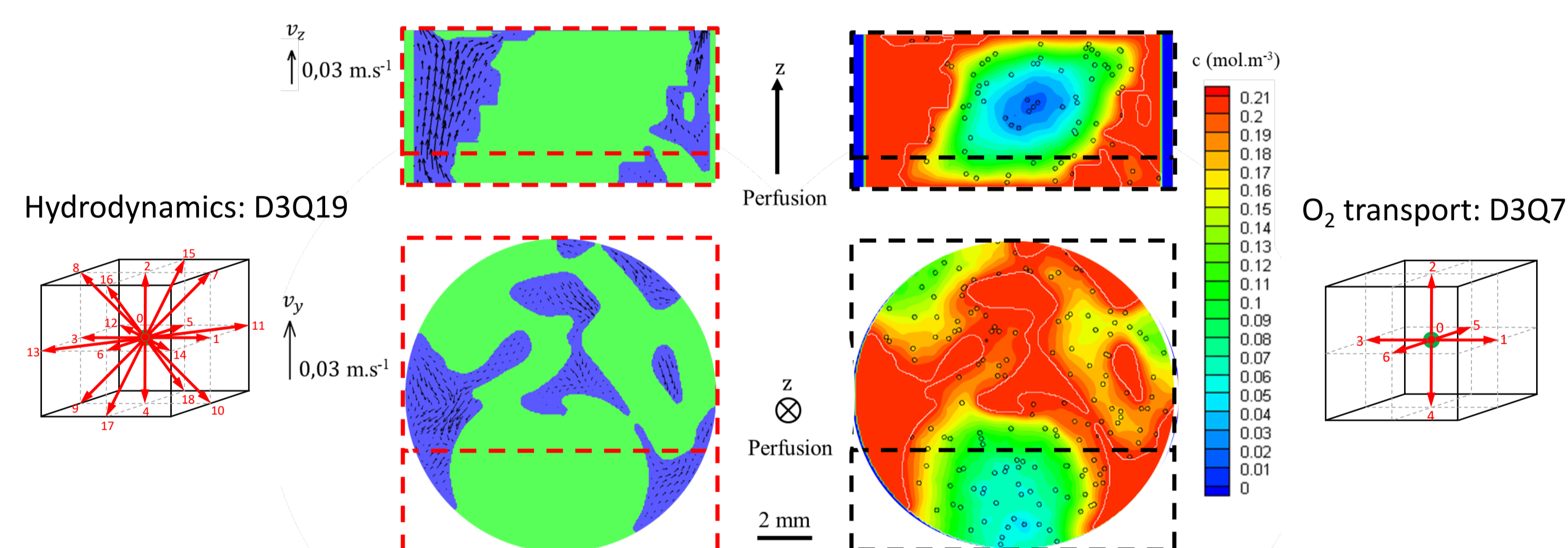
Green: viable cells
Red: dead cells

Bioreactor modeling

- $\vec{\nabla} \cdot \vec{v} = 0$
 - $\rho \frac{\partial v_\alpha}{\partial t} + \rho \vec{v} \cdot \vec{\nabla} v_\alpha = \rho g_\alpha - \frac{\partial p}{\partial x_\alpha} + \mu \Delta v_\alpha \quad \alpha = x, y, z$
 - $\frac{\partial C_{O_2}}{\partial t} + \vec{v} \cdot (C_{O_2} \vec{v}) = -D_{O_2} \Delta C_{O_2} + \Omega_{O_2}$
- Hydrodynamics**
- Species (O₂) 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⁻¹)
 - C_{O_2} : O₂ concentration (mol.m⁻³)
 - D_{O_2} : O₂ diffusion coefficient (3 10⁻⁹ m².s⁻¹ in the fluid, 1.6 10⁻⁹ m².s⁻¹ in the hydrogel)
 - ϑ : cell volume (3000 μ m³)
 - V_{max} : maximum O₂ consumption rate (4 10⁻¹⁷ mol.cell⁻¹.s⁻¹)
 - K_M : Michaelis-Menten constant (6 10⁻³ mol.m⁻³)

Hydrodynamics and oxygen transport

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



- Maximum fluid interstitial velocity reaches **27 mm.s⁻¹**
- 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 O₂ transport using **Lattice Boltzmann Methods (LBMs)**
 - Easily handle complex geometries such as porous media
 - Use « directly » the 3D images acquired by μ CT⁶, MRI, or OCT

LBM principle

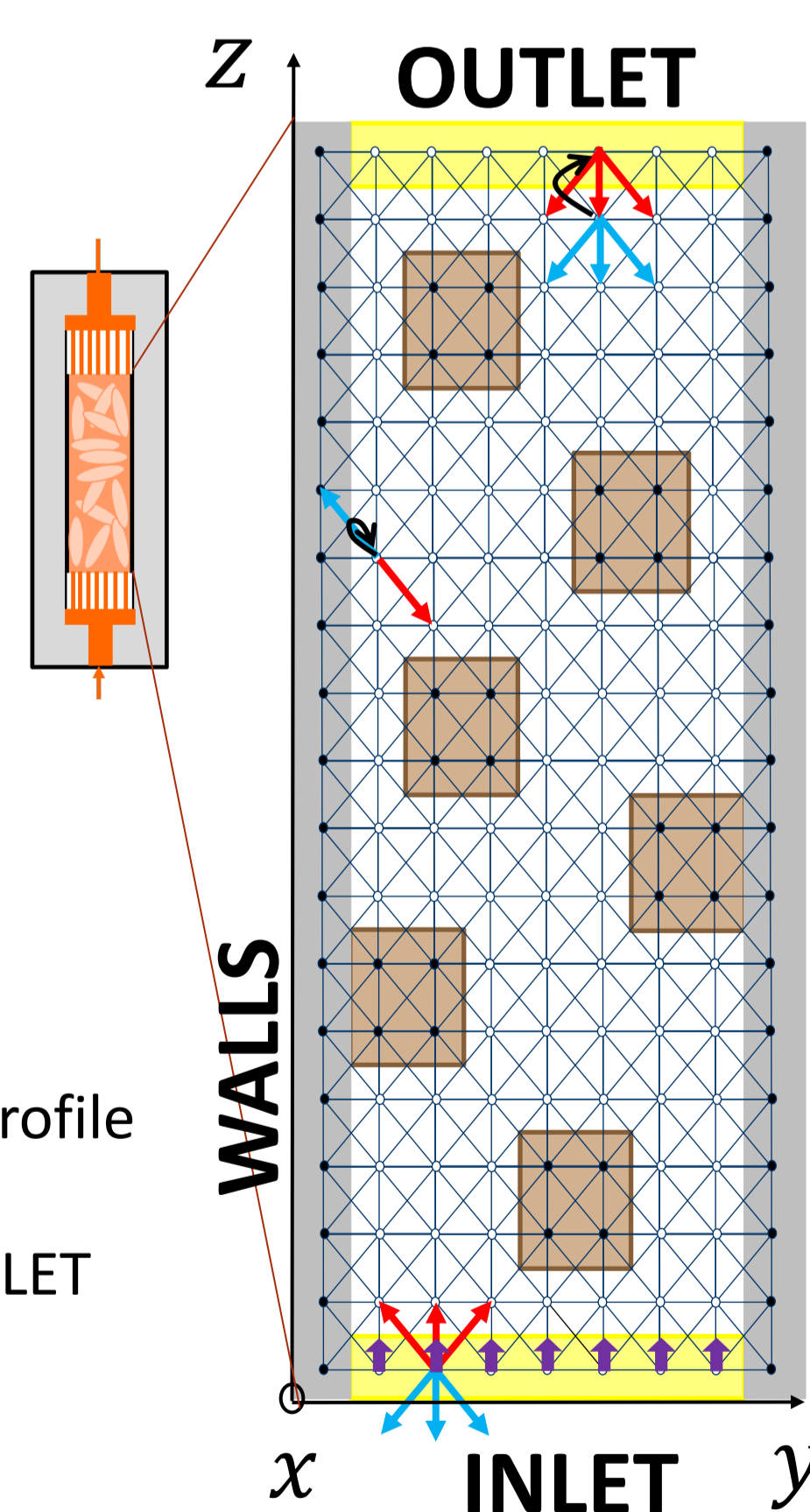
- 3D space mapped on a regular lattice
- Fluid (resp. species) represented by a population of fictitious particles with a discrete and finite velocity distribution (Q particle velocity vectors)
- Particles constrained to move along the lattice
- At each time step, dynamics divided into two stages
 - Propagation of the particles from node to node
 - Collisions between particles located at the same node
- Pressure p , hydrodynamic velocity \vec{v} , (resp. O₂ concentration C_{O_2}) obtained by taking appropriate moments of the velocity distribution function

Boundary conditions

- Flat and constant fluid velocity (resp. O₂ concentration) profile at the **INLET**
- Zero velocity (resp. O₂ concentration) gradient at the **OUTLET**
- No-slip condition (resp. zero-flux) at impermeable **WALLS**: bounce-back rule

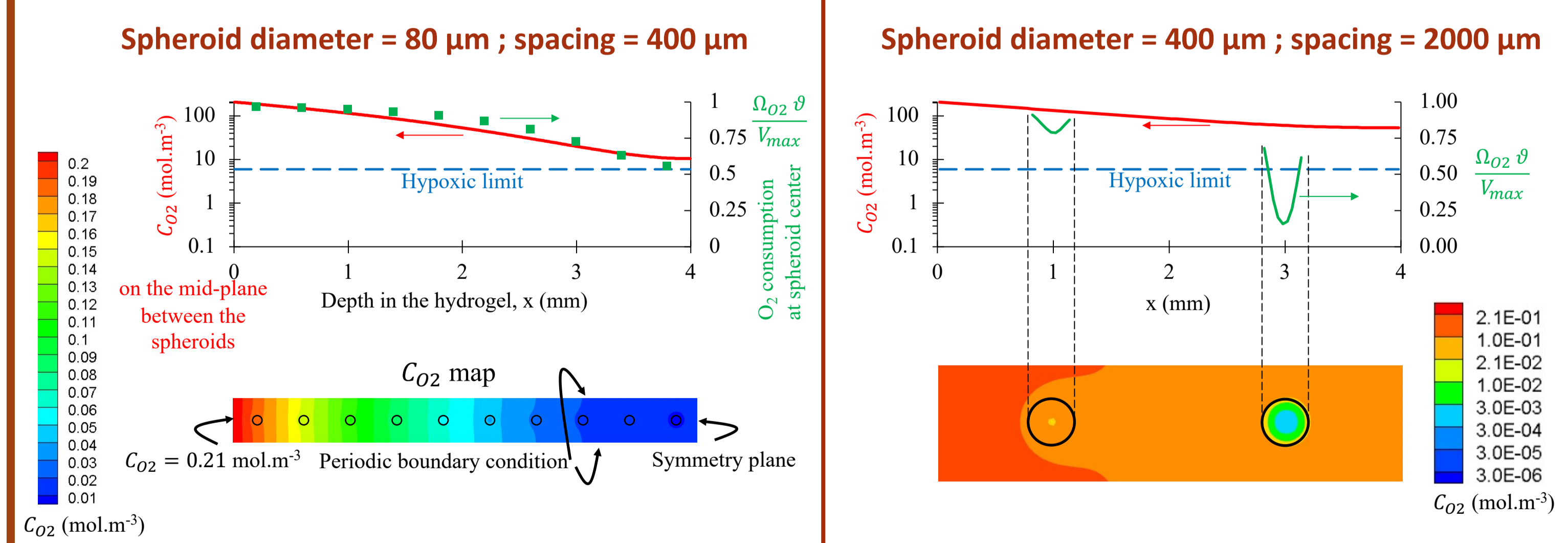
Characteristics

- Two-relaxation-time collision operators (TRT)^{7,8}
- $Q = 19$ particle velocities for the hydrodynamics → D3Q19
- $Q = 7$ particle velocities for the species transport → D3Q7



At the scaffold scale

- Simulation of O₂ transport with a higher spatial resolution (10 μ 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

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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