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

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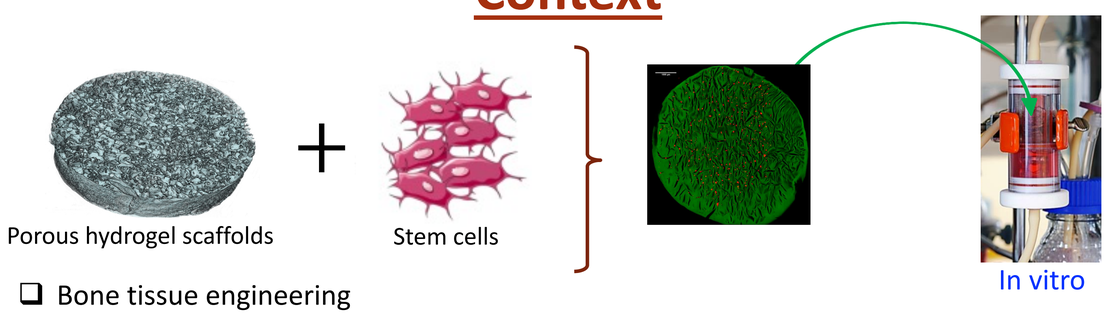
Context

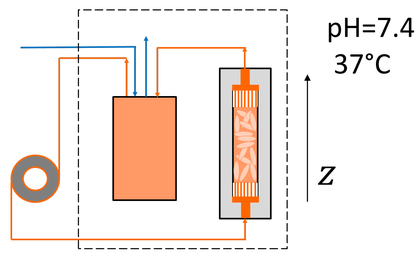


- Bone tissue engineering
  - In vitro model of bone reconstruction
- Porous scaffolds seeded with MSCs cultured in a perfusion bioreactor[1]
  - Pullulan/dextran porous hydrogel scaffolds[2,3] → 3D microenvironment / → Spontaneous formation of spheroids[4]
  - Perfusion flow: long-term delivery of oxygen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

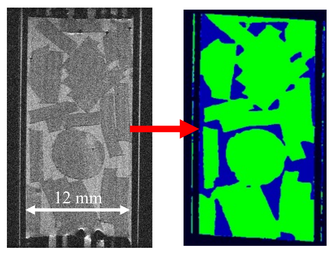
Aim: Develop a bioreactor digital twin to determine the chemical/mechanical microenvironment seen by the cells

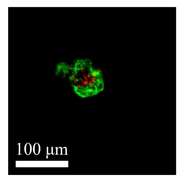
Custom-made perfusion bioreactor

- 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 CO_2-bubbling
- Perfusion rate: 10 mL.min^{-1}
- 1 to 3 weeks of dynamic culture



- 3D geometry of the stack
  - 7T MRI, resolution: 55 µm
  - 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 µm
  - Live/Dead assay → viability of 80%





Green: viable cells
Red: dead cells

Bioreactor modeling

$$(1)\ \vec{\nabla}.\vec{v} = 0$$

$$(2)\ \rho\frac{\partial v_\alpha}{\partial t} + \rho\vec{v}.\vec{\nabla}v_\alpha = \rho g_\alpha - \frac{\partial p}{\partial \alpha} + \mu\,\Delta v_\alpha \qquad \alpha = x, y, z$$

Hydrodynamics

$$(3)\ \frac{\partial C_{O2}}{\partial t} + \vec{\nabla}.(C_{O2}\vec{v}) = -\mathcal{D}_{O2}\Delta C_{O2} + \Omega_{O2}$$

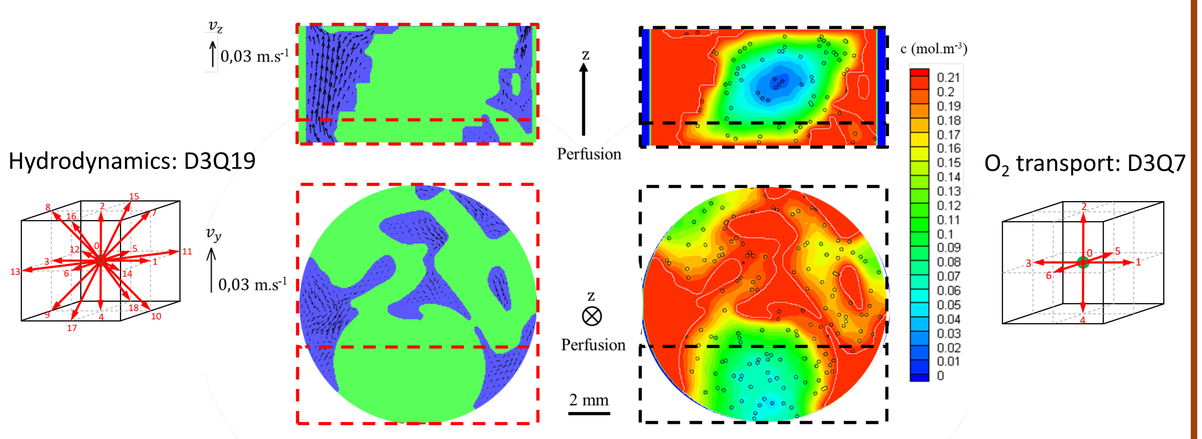
$$\Omega_{O2} = \begin{cases} \dfrac{V_{max}}{\vartheta}\dfrac{C_{O2}}{C_{O2}+K_M} & \rightarrow O_2 \text{ consumption by spheroids} \\ 0 & \rightarrow \text{anywhere else} \end{cases}$$

Species (O_2) transport

- $\vec{v}$: Fluide velocity (m.s^{-1})
- C_{O2} : O_2 concentration (mol.m^{-3})
- $\mathcal{D}_{O2}$: O_2 diffusion coefficient ($3\ 10^{-9}$ m^2.s^{-1} in the fluid, $1.6\ 10^{-9}$ m^2.s^{-1} in the hydrogel)
- ϑ : cell volume (3000 µm^3)
- V_{max} : maximum O_2 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 µm-diameter spheroids
- Each spheroid represented by eight 55 µm-voxels
- Number of spheroids adjusted to obtain about 100,000 cells per scaffolds



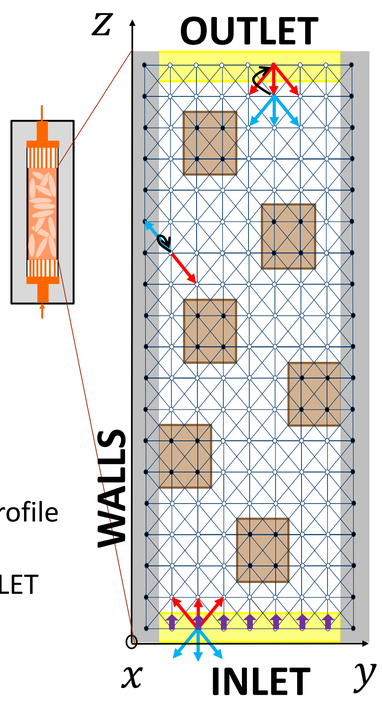
→ Maximum fluid interstitial velocity reaches **27 mm.s^{-1}**
→ Wall shear stress from **0.1 mPa to 100 mPa** , average value of **25 mPa**
→ **98%** of the total hydrogel volume is **non hypoxic** (criterion: $C_{O2} > K_M$)
→ Consistent with the viability assessment of the cells

LBM scheme and boundary conditions

- Simulation of hydrodynamics and O_2 transport using **Lattice Boltzmann Methods** (LBMs)
  - Easily handle complex geometries such as porous media
  - Use « directly » the 3D images acquired by µCT[6], 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_2 concentration C_{O2}) obtained by taking appropriate moments of the velocity distribution function



Boundary conditions

- Flat and constant fluid velocity (resp. O_2 concentration) profile at the INLET
- Zero velocity (resp. O_2 concentration) gradient at the OUTLET
- No-slip condition (resp. zero-flux) at impermeable WALLS: bounce-back rule

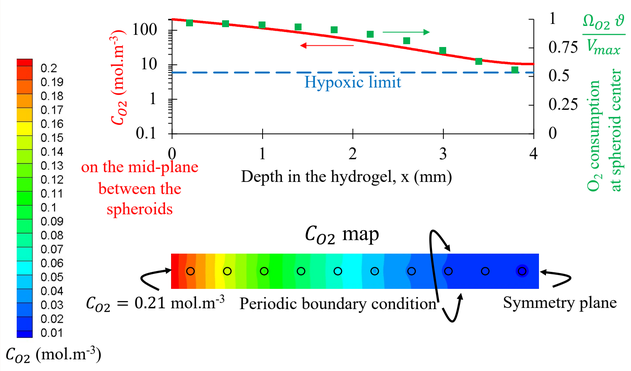
Characteristics

- 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

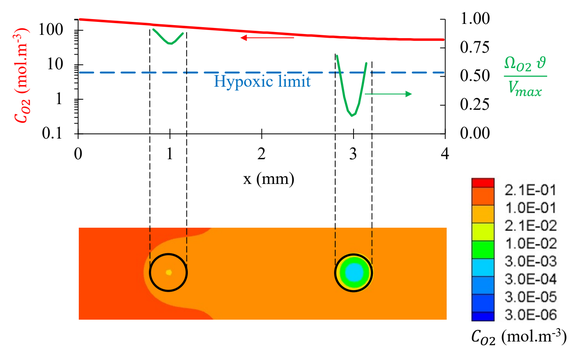
- Simulation of O_2 transport with a higher spatial resolution (10 µm)
- Model configuration: spheroids of same diameter evenly spaced in a hydrogel scaffold
- 100,000 cells per scaffold

Spheroid diameter = 80 µm ; spacing = 400 µm



→ no hypoxia up to 4 mm depth

Spheroid diameter = 400 µm ; spacing = 2000 µm



→ 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