LARGE EDDY SIMULATION OF THE HYDRODYNAMICS INSIDE MINI-BIOREACTORS DESIGNED FOR STEM CELL CULTURE

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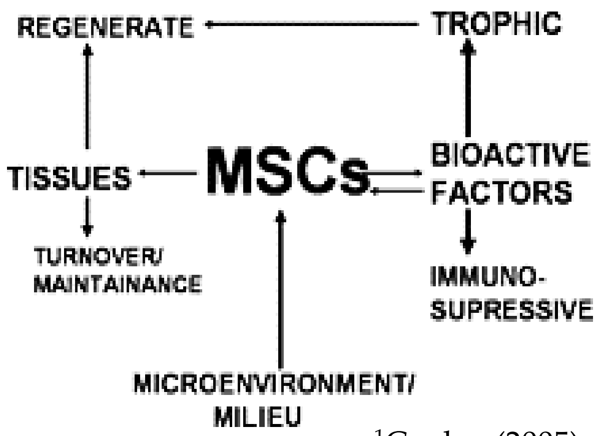
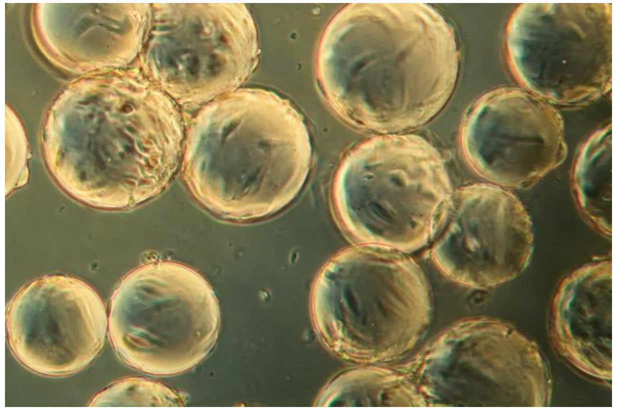
Mesenchymal stem cell culture and medical applications



[1]Caplan (2005)



hMSC culture on microcarriers

Mesenchymal stem cells (MSC) offer very promising medical applications[1]

- **Regeneration of tissues** (bone, cartilage, tendon, ligament, etc…)
- **Cell therapy** (heart diseases, cancer, diabetes, etc…)

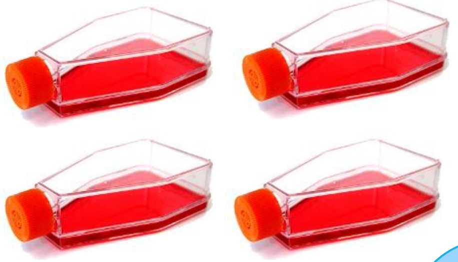
MSC culture present **specificities** which should rule the culture bioprocess:

- Cells = products
- **Scarcity** in organism
- Sensitive to their biochemical niche and hydromechanical stress (**stemness, differentiation capacity**).
- MSC are most commonly cultured in 2D static systems (T-flasks) and seldom in mixed and sparged bioreactors (of a few liters).
- In mixed bioreactors, MSC are successfully cultured on **microcarriers**[2,3,4,5]

[1]Caplan (2005); [2]Rafiq et al. (2013); [3]Nienow et al.,(2014); [4]Sart et al. (2010); [5]Hewitt et al. (2011)

Introduction

The challenge of the scale-up of MSC production process



Development of a minibioreactors platform for process scale-up

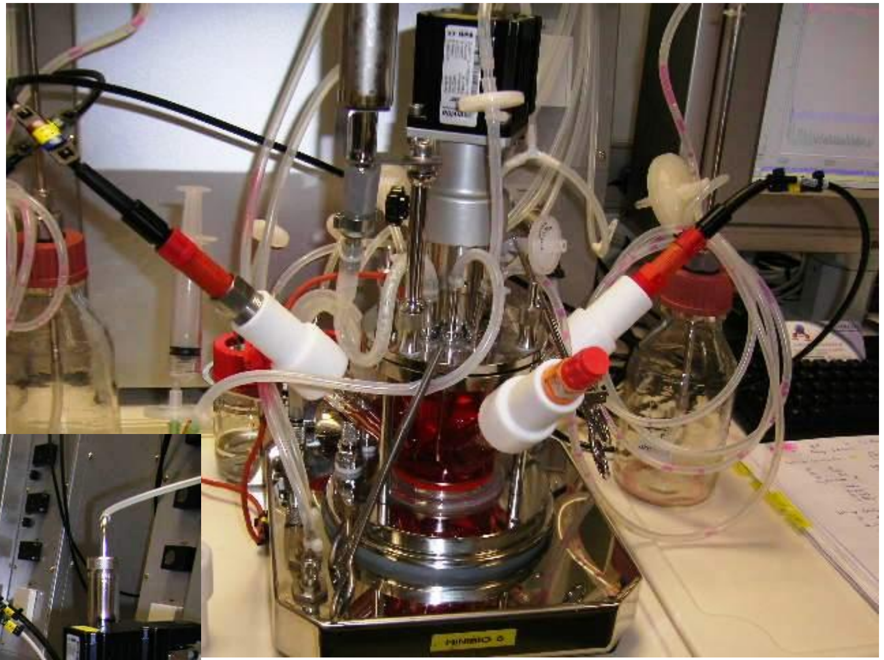
- Reduction of culture medium volume
- Scale-down of hydrodynamics and biochemical environment
- Lower Reynolds numbers ?

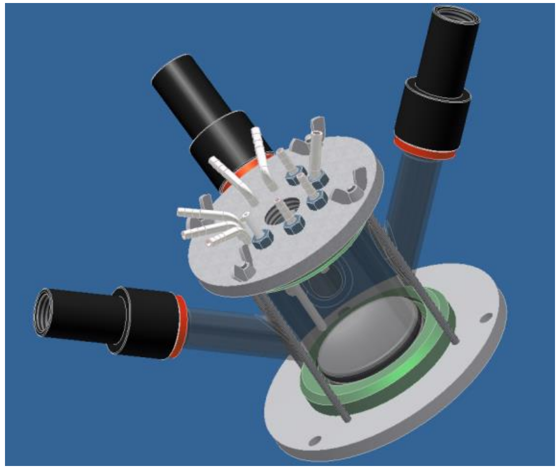
Global hydrodynamics (power dissipation, mixing and circulation times)
Local hydrodynamics (temporal distribution of turbulent dissipation rates)

Biochemical environment
Critical agitation rate for microcarrier suspension
Hydrodynamic damage on microcarrier
Collisions between microcarriers

Mini-bioreactors platform

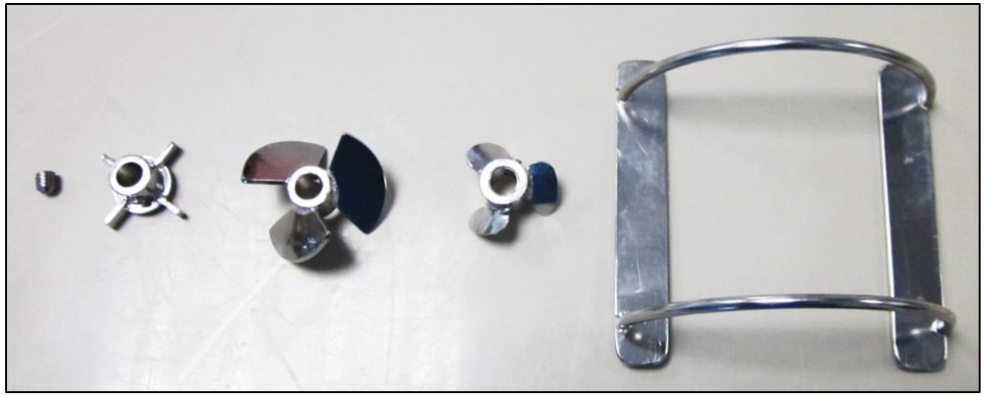
- 6 Glass-made, sterilizable vessels
  (V_T = 250 mL, V_{min} = 50 mL).
- **Geometric similarity** with standard vessels (Diameter T = 60 mm).
- Implementation of **three standard-sized probes** (among O_2 , pH, biomass, CO_2).
- Temperature regulated with heated bottom.
- Full **independent regulation** of culture parameters (pH, O_2, T°) with dedicated software and related equipment.
- Developed in collaboration with Global Process Concept (GPC, La Rochelle, France)





Mini-bioreactors platform

Agitation



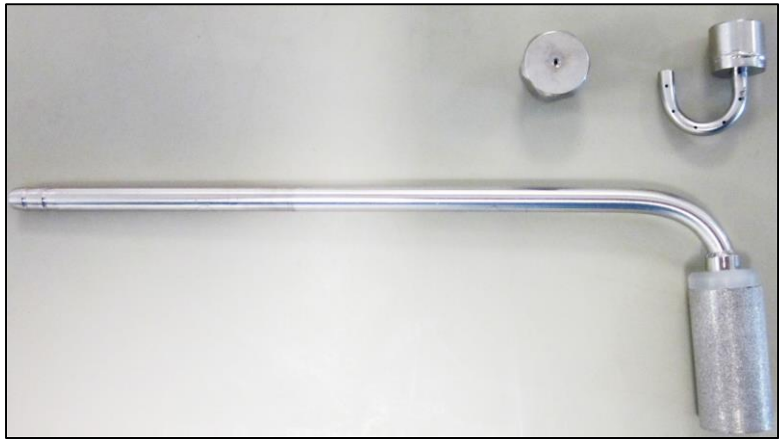
Agitation can be ensured by:

- A 4 bladed - **Rushton turbine** (D / T = 0.33)
- An Up-pumping (MPU) and Down-pumping (MPU) **Marine Propeller** (D / T = 0.33)
- An Up-pumping (EEU) and Down-pumping (EED) **'ear-elephant' impeller** (D / T = 0.45)

and:

- 2 removable vertical baffles.
- $20 < N < 500$ rpm

Aeration



Gas sparging (air / O_2 / N_2 / CO_2) can be ensured by :

- An Inox sintered cylinder.
- A single orifice sparger.
- A multi-orifice sparger.

Surface aeration.

CFD numerical simulations

Turbulence modelling in bioreactors is a key issue (mass transfer, mixing, hydromechanical stress)

1. Reynolds Averaged Navier-Stokes (RANS) approach

- Steady-state simulations in Moving Reference Frame (MRF).
- Model all the turbulence length scales.
- Turbulence closure model.
- LES simulation mesh building.
- “Screening” simulation.

2. Large-Eddy Simulation (LES)

- Transient simulation with Sliding Mesh approach.
- Simulates the largest scale of turbulence and model the universal small scales.
- Turbulence subgrid model (Smagorinsky…)
- Finer description but higher computational cost

Large Eddy Simulation

- LES separates the velocity field into a resolved and sub-grid part.
- The resolved part of the field represent the "large" eddies, while the subgrid part of the velocity represent the "small scales" whose effect on the resolved field is included through the subgrid-scale model.
- Formally, one may think of filtering as the convolution of a function with a filtering kernel G:

$$\bar{u_i}\,(\boldsymbol{x}) = \int G(x - \boldsymbol{\xi})u(\boldsymbol{\xi})d\boldsymbol{\xi}$$

With the velocity decomposition :

$$u_i = \bar{u_i} + u_i'$$

Substituting this decomposition in the Navier-Stokes and assuming an incompressible flow:

$$\frac{\partial \overline{u_i}}{\partial t} + \overline{u_j}\frac{\partial \overline{u_i}}{\partial x_j} = -\frac{1}{\rho}\frac{\partial \bar{p}}{\partial x_i} + \frac{\partial}{\partial x_j}\left[(\nu + \nu_t)\frac{\partial \overline{u_i}}{\partial x_j}\right]$$

Large Eddy Simulation

Turbulent viscosity modelling by the Smagorinsky-Lilly model

$$\nu_t = (C_s\Delta)^2|\bar{S}|$$

$$\bar{S} = \sqrt{2 \cdot S_{ij} \cdot S_{ij}}$$

Filter width is given by the local mesh size

$$\Delta = (Volume)^{1/3}$$

$$\overline{S_{ij}} = \frac{1}{2}\left(\frac{\partial \bar{u}_i}{\partial x_j} + \frac{\partial \bar{u}_j}{\partial x_i}\right)$$

The constant C_S has usually the value 0.1 – 0.2

Is the rate-of-filtered strain tensor

CFD numerical simulations

Most important considerations in animal cell culture arise from the prediction of the occurrence of « **shear damage** ».

➢ Description of **turbulent dissipation rates** fields is necessary to access this information

1. Reynolds Averaged Navier-Stokes (RANS) approach

The turbulent dissipation rate is directly calculated by the turbulence model

2. Large-Eddy Simulation (LES)

The turbulent dissipation rate is obtained from the velocity field

$$\varepsilon = 2 \cdot (\nu + \nu_T) \cdot S_{ij} \cdot S_{ij}$$

A common validation consists in comparing required and dissipated mechanical power

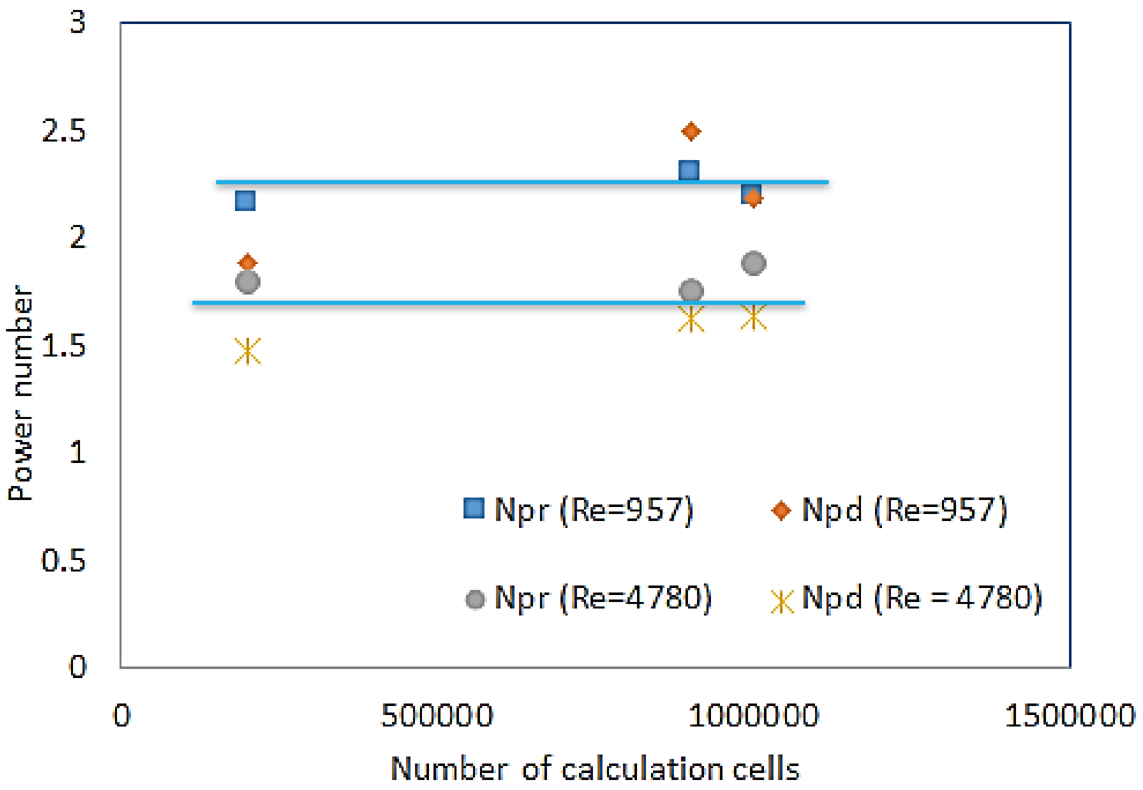
$$Np_{required} = \frac{2\pi \cdot N \cdot C}{\rho_L \cdot N^3 \cdot D^5} \longleftrightarrow Np_{dissipated} = \frac{< \varepsilon > \cdot V_L}{N^3 \cdot D^5}$$

CFD numerical simulations

Design of calculation mesh for RANS simulations

For MRF simulations, various mesh designs were compared and discriminated by comparison of required and dissipated power numbers.

Fair impact of mesh design on Np : for **RANS simulations, the 0.9 M cells mesh** was chosen for the rest of the study



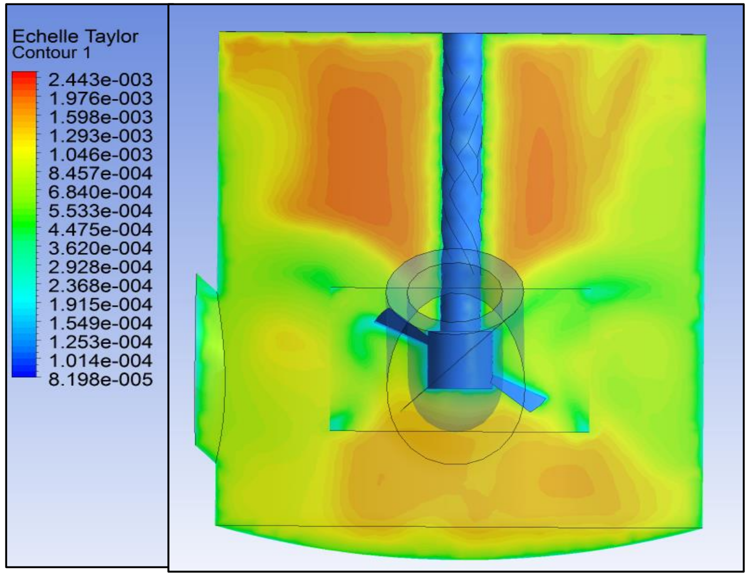
Impact of number of calculation cells on impeller power number (RANS k-ε model, Ear-Elephant)

CFD numerical simulations

Mesh design

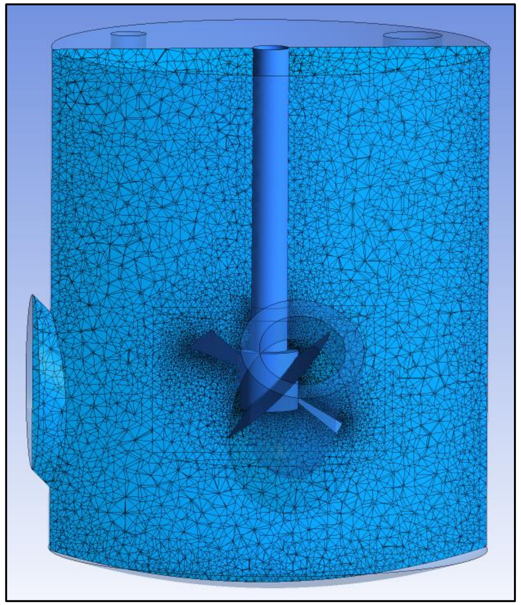
- For SM - LES simulations, the mesh was built following the estimation of Taylor microscale (isotropic turbulence).

$$\lambda = \sqrt{\frac{15 \cdot \nu \cdot \overline{u'^2}}{\varepsilon}} \simeq \sqrt{\frac{10 \cdot \nu \cdot k}{\varepsilon}}$$



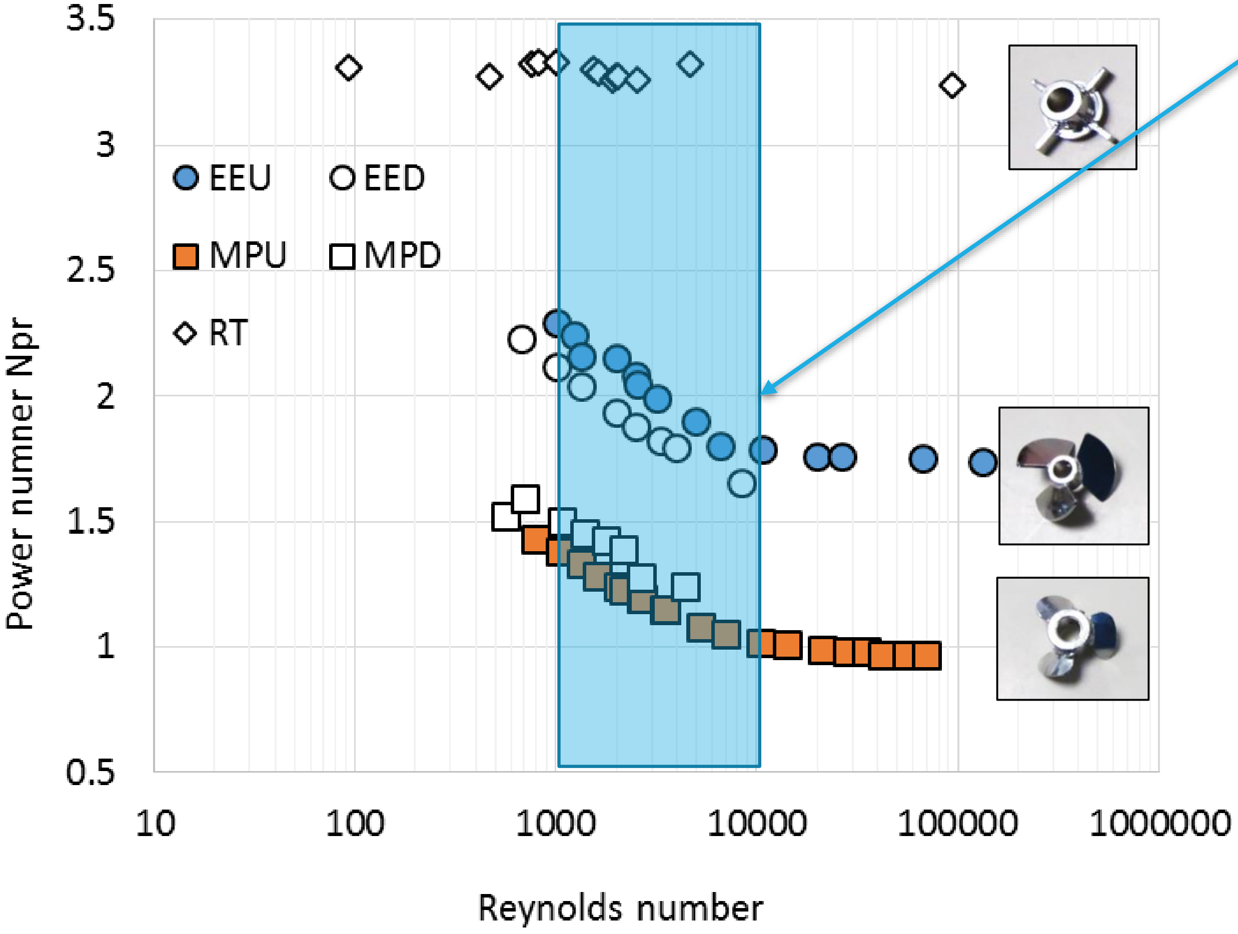
MRF Taylor microscale simulation ←

LES mesh →



- For the LES simulations, the mesh was built using 1.8 M of tetrahedral cells.
- 50 µm < characteristic mesh length < 1.2 mm
- Each time step Δt corresponded to 0.5 ° of impeller rotation.
- Calculation time was around three weeks for a 30 s real time simulation.

Simulation results



Range of Reynolds numbers typically used for MSC culture in minibioreactors[1]

Power curves of the various impellers inside the minibioreactor (CFD-RANS k-ε realizable, 0.9 M cells)

Comparison of turbulence closures

Turbulence closure	Npr	Npd	Ratio Npd / Npr
$k-\varepsilon$ standard	2.3	3.0	130 %
$k-\varepsilon$ realizable	2.1	2	93 %
Transition SST	2.2	1.9	86 %
$k-\varepsilon$ RNG	2.2	3.0	136 %
LES, dynamical Cs	2.0	1.8	90 %
LES, Cs = 0.2[2]	2.2	2.0	91 %
LES, Wale	2.3	3.4	147 %

EEU impeller, Re = 1000

Nienow (2014); [2]Delafosse et al., 2008

Simulation results

RANS $k - \varepsilon$ realizable and LES (Smagorinsky, Cs = 0.2) have been retained for detailed comparisons

Validity of the turbulence closure models used ?

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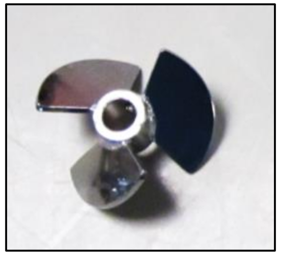
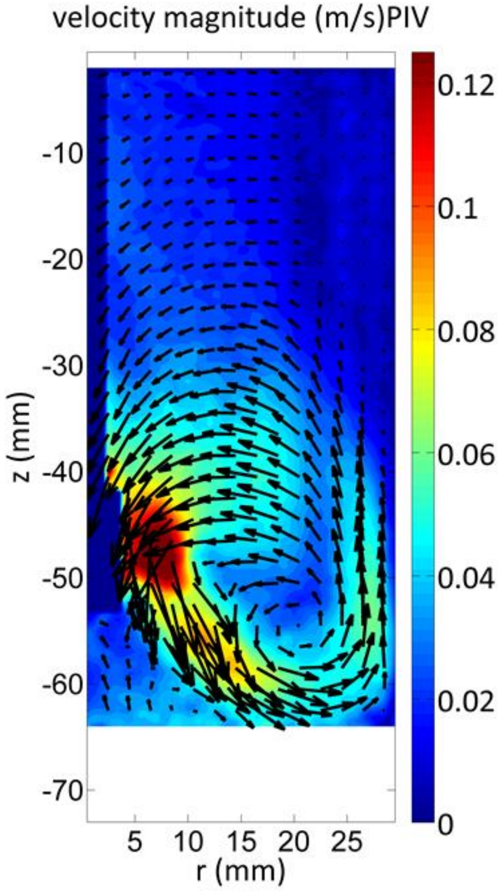
EEU impeller, Re = 1000

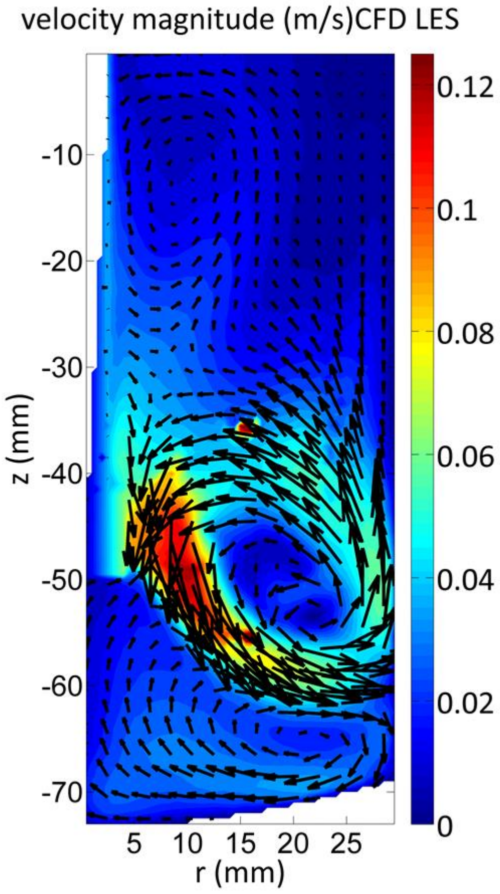
Simulation results

Comparison of mean velocity fields
Elephant-Ear, down-pumping, Re = 2000, (θ = 18°)

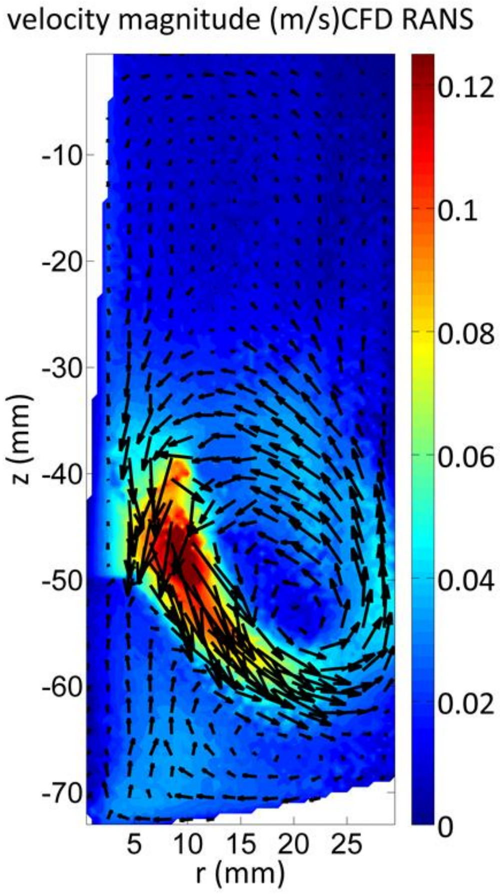




PIV measurements



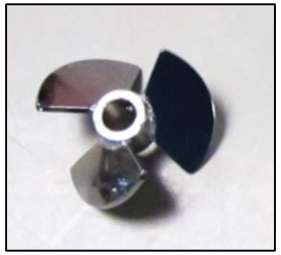
CFD - LES

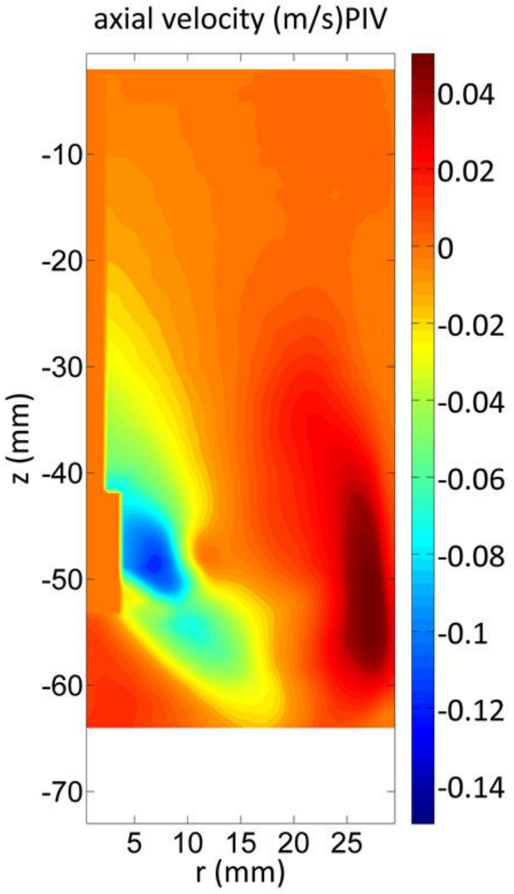


RANS k-ε realizable

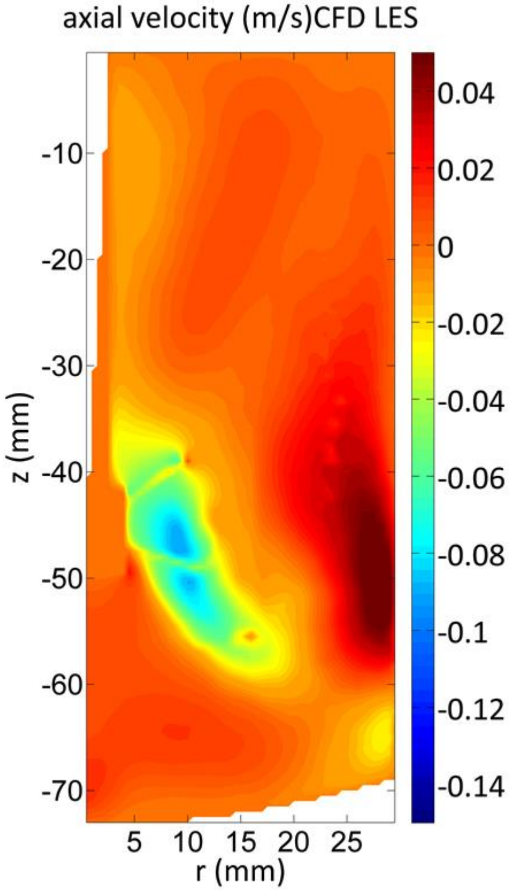
Simulation results

Comparison of mean axial velocity fields
Elephant-Ear, down-pumping, Re = 2000, (θ = 18°)

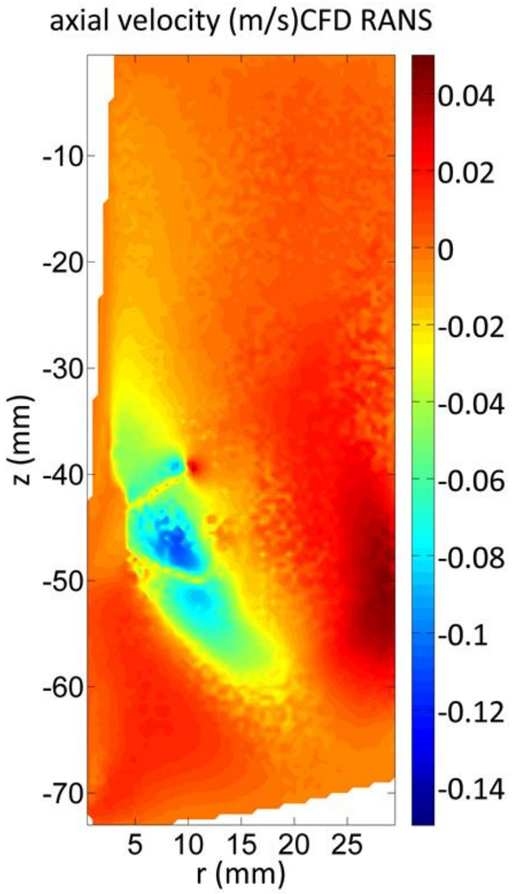




PIV measurements



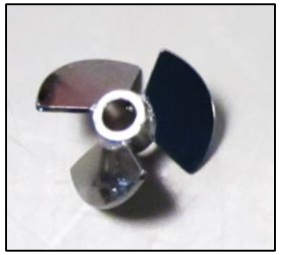
CFD - LES

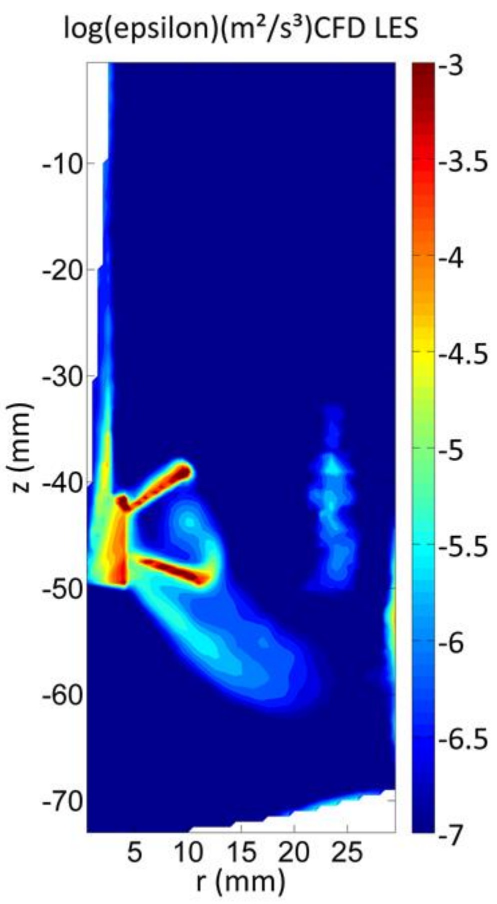


RANS k-ε realizable

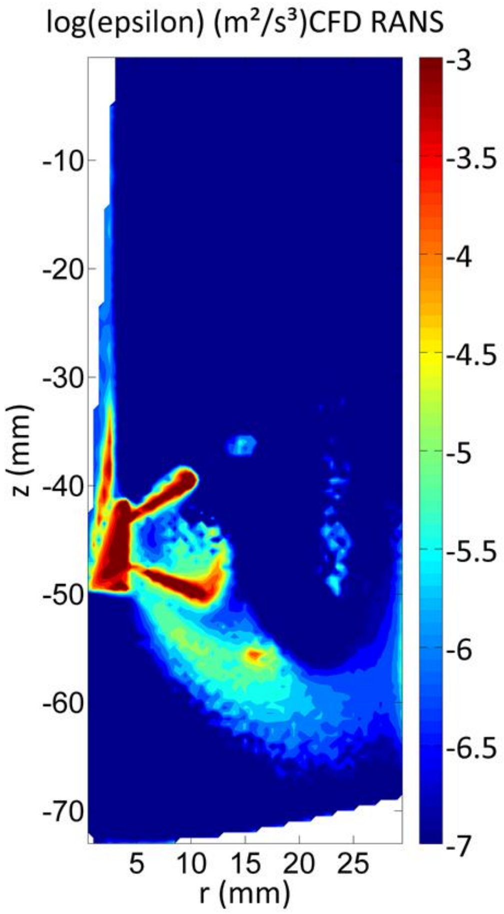
Simulation results

Comparison of turbulent dissipation rate fields
Elephant-Ear, down-pumping, Re = 2000, (θ = 18°)





CFD - LES

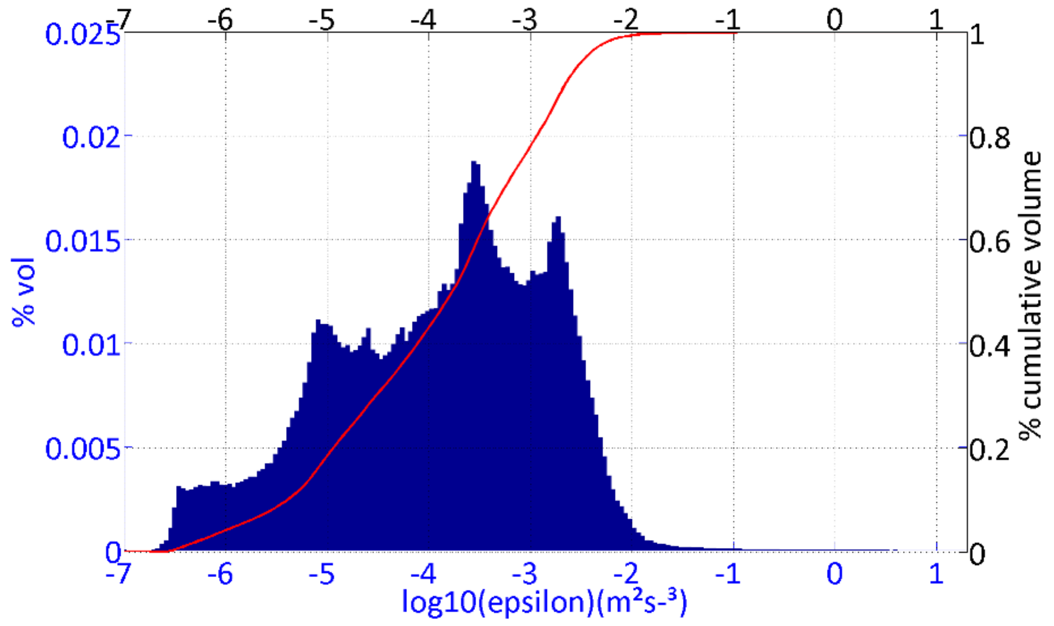


RANS k-ε realizable

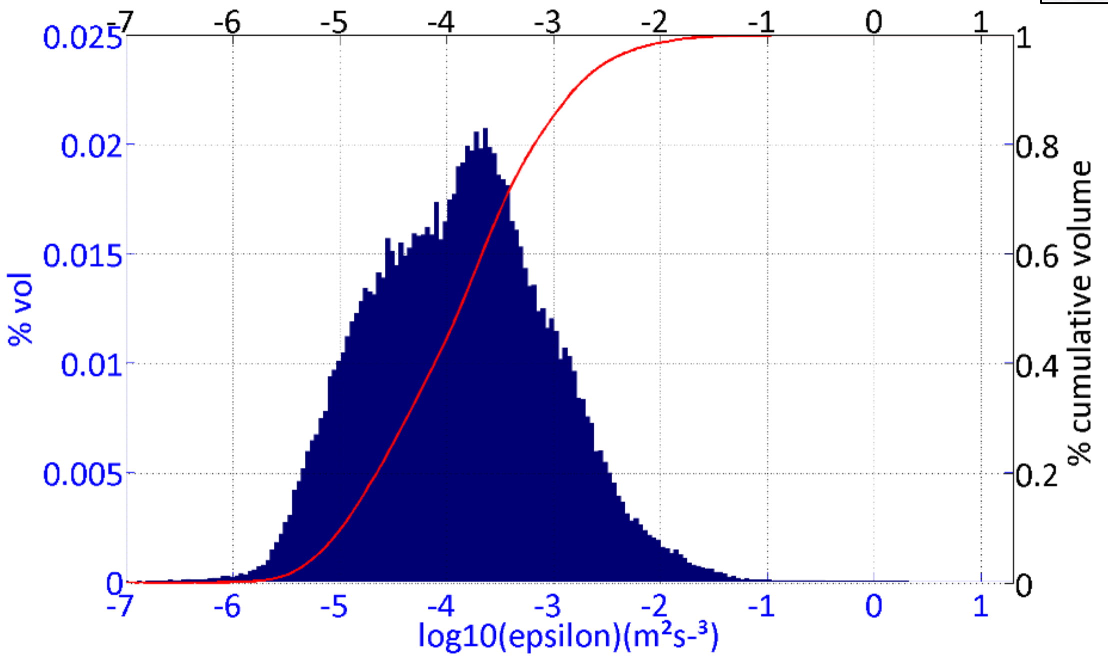
Heterogeneity of the dissipation rate : what about its statistical distribution ?

Turbulent dissipation rate (TDR) distributions

(Ear Elephant Down-pumping, Re = 2000)



RANS k-ε realizable

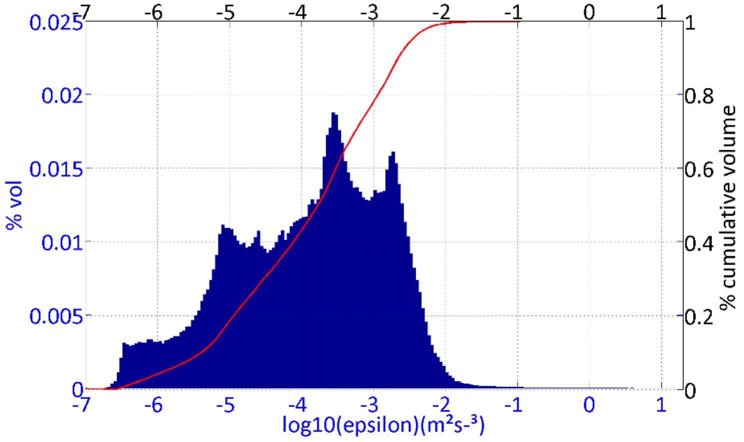


LES (Cs = 0.2)

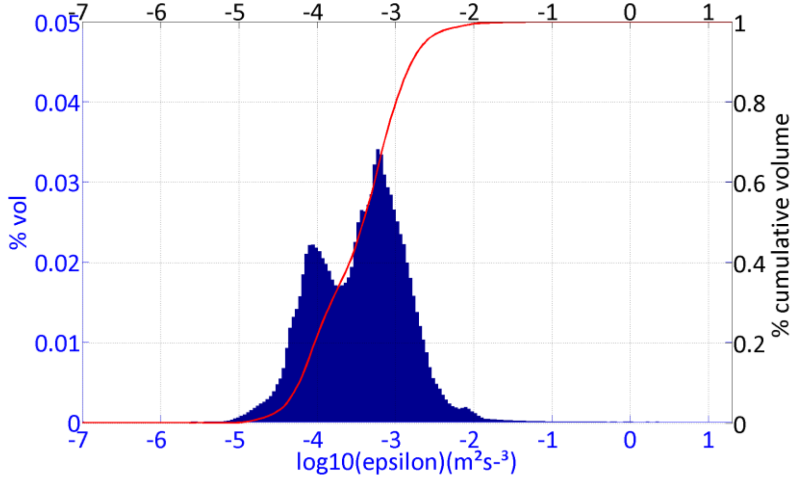
LES simulations predict TDR distributions closer to a normal distribution

Simulation results

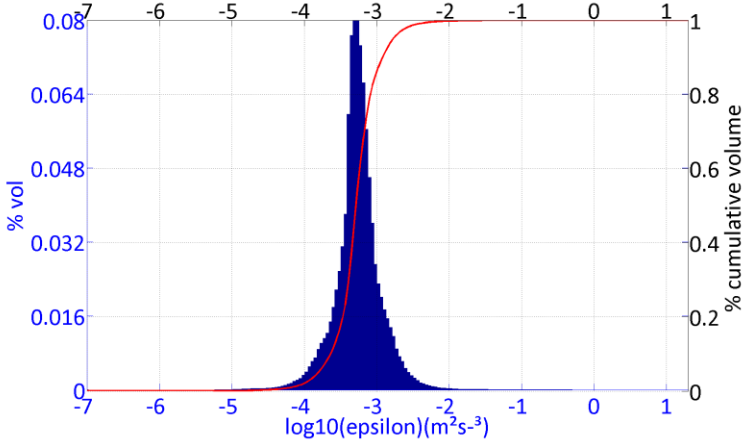
Scale-up of turbulent dissipation rate distributions at constant *P* / *V*
(CFD, RANS k-ε realizable, Ear Elephant Down-pumping)



V_L = 200 mL
Re = 2000



V_L = 1.2 L
Re = 4900



V_L = 20 L
Re = 17000

From mini- to pilot (production) scale, the dispersion of ε becomes narrower, justifying the use of statistical distribution for bioreactor scale-down studies.

Simulation results

Scalability of the bioreactor at the just-suspended state.

Impeller	Reynolds number		P / V (W m^{-3})	
	200 mL	20 L	200 mL	20 L[1]
 EE – down	1,000	7,500	0.15	0.3
 MP – down	2,400	13,000	0.14	0.2

Data were obtained for a suspension of 9 g L^{-1} of Cytodex-1 microcarriers. Values of *P* / *V* in the 20-L bioreactor were measured from torque on the impeller.

At the microcarrier just-suspended state

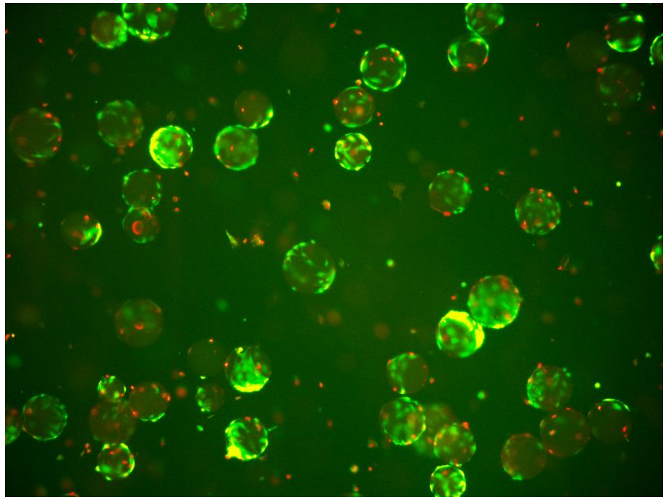
- Power dissipation was maintained from 200 mL to 20 L
- Reynolds number was lower in minibioreactor questioning flow regime

1. Collignon et al. (2010)

Conclusion and perspectives

- Design and scale-up of a novel flexible mini-bioreactor platform for MSC culture.
- RANS and LES simulations validity have been compared.
- Scalability of the performance by comparison with 2 and 20 L bioreactor in geometrical similarity.

- Integration of microcarrier suspension in particle hydromechanical stress characterization.
- Impact of impeller and reactor design and agitation on MSC expansion performance.



Successful hMSC culture on Cytodex-1 microcarrier in minibioreactor (EEU, 75 rpm)