CFD Large Eddy Simulation of the hydrodynamics of stirred mini-bioreactors operating with stem cell culture mixing conditions

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CONTEXT: The expansion of hMSC adhered on microcarriers is a proven technology to allow the production of quantitatively (cell density and growth rate) and qualitatively (preservation of cell stemness) the amounts of cells required for clinical applications.

STUDY: In that context of process optimization, **a platform of 6 parallel 250 mL stirred minibioreactors** have been designed and fully equipped for hMSC cultivation on microcarriers. **The local hydrodynamics** in these bioreactors have been **characterized using CFD simulations and experimental measurements.**

GOALS:
1) **Evaluation of the flexibility** of the platform for extensively **studying the coupling between hydrodynamics and hMSC physiological response** (growth, stemness) **by changing agitation conditions**.
2) **Evaluation of the scale down ability of platform** as prediction tool of the hMSC behavior in larger bioreactor scale.

Reynolds Averaged Navier-Stokes approach (RANS)

- Steady-state simulation
- Model all the turbulence length scales
- 0.9 M of tetrahedral cells
- Rapid computation (24 hours)

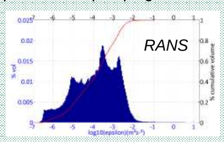
➔**Screening tool**

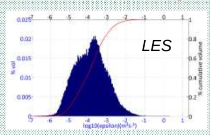
Large Eddy Simulation (LES)

- Transient simulation
- Simulates the largest turbulence scale and model only the small scales
- 1.8 M tetrahedral cells: <50 µm -1.2 mm>
- Long computation (2 weeks)

➔ **Reliable analysis tool for transient flow regime**

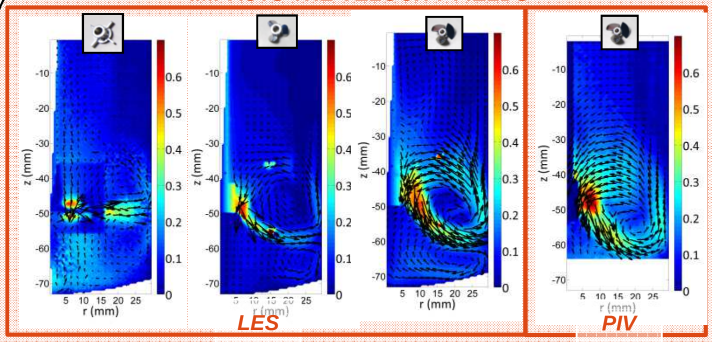
Ear Elephant down-pumping, Re = 2000





Volumetric distribution of turbulent dissipation rate (TDR)
➔ ***Same mean value***
➔ ***Discrepant numerical distribution***

IMPACTS THE VELOCITY FIELDS



The **structure of normalized time averaged velocity field** simulated by LES and validated by stereo-PIV measurements (right) strongly varies with impeller type and impeller pumping direction (not shown).

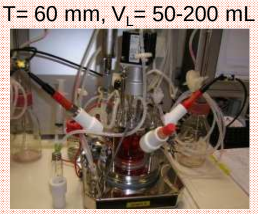
Variable passage frequencies in the impeller :

$$f_p(s^{-1}) = \frac{N_{QP} N d^3}{V_L} = \begin{cases} 0{,}0384\,N \text{ for } EE \\ 0{,}0120\,N \text{ for } Marine \\ 0{,}0068\,N \text{ for } RT \end{cases}$$ with V_L=0.2 L

CUSTOM-MADE HYDRODYNAMICS IN A FLEXIBLE PLATFORM OF 6 PARALLEL STIRRED MINIBIOREACTORS

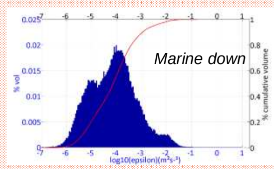
Full automatic regulation :
- lateral pH and pO_2 probes
- Hot and cooling bottom
- Aeration in volume or by liquid surface
- Continuous or intermittent agitation (**N: 20-500 rpm**)

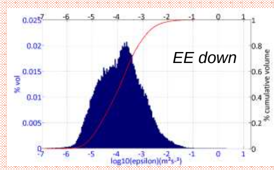
Glass-made, sterilizable vessel
T= 60 mm, V_L= 50-200 mL



Rushton Turbine (D/T=0.33)
Up/down EE impeller (D/T=0.45)
Up/down Marine propeller (D/T=0.33)

IMPACTS THE TURBULENT DISSIPATION RATE DISTRIBUTION

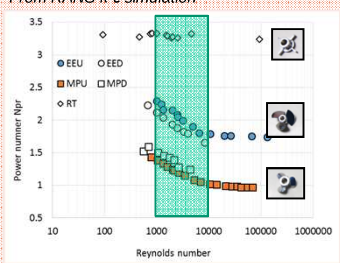




For a same P/V, the shape and the spread (i.e. maximal value) of **turbulent dissipation rate volumetric distribution** strongly **depends on impeller design**.

SCALING DOWN BIOREACTORS: A CAREFULL PROCEDURE

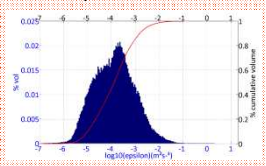
From RANS k-ε simulation



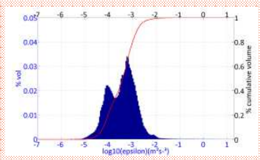
➔ Reynolds number of **transient flow** regime in small bioreactor while turbulent flow in larger scale.

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

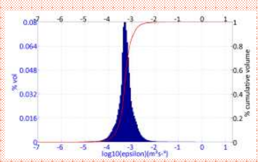
Scale-up of TKE at constant P/V



EE down LES V_L=0.2 L Re=2000



EE down RANS V_L=1.2 L Re=4900



EE down RANS V_L=20 L Re=17000

IMPACTS MICROCARRIERS SUSPENSION QUALITY

Impeller	Just-suspended agitation rate (rpm)	P/V (W m⁻³)
EE down	75	0.29
EE up	130	1.52
RT	163	1.05
Marine down	140	0.30
Marine up	340	3.54

(Data for Cytodex-1, 8 g L^{-1})

EE down

Marine down

EE up

Marine up

Rushton Turbine

For down-pumping impellers and RT, power dissipation is lower at N_{js} but quality suspension is weaker

CONCLUSIONS AND PERSPECTIVES

- A flexible platform of 6 minibioreactors was build for studying the coupling between hydrodynamics and hMSC behavior via the generation of a characterized custom-made flow.

➔ *Enlarge custom-made flow capacity by modifying liquid height, impeller implementation height or impeller diameter*

- Scale down ability is limited when conserving geometric similarity and a constant P/V

➔ *Opportunity to rethink scale-up strategies*