# A BIOPROCESSING APPROACH TO CONTROL AND DESCRIBE LARGE BIOPRINTED TISSUE MICROENVIRONMENT IN PERFUSION BIOREACTORS

**Elliott Cowles**[1], Céline Thomann[1], Lucie Essayan[1], Denis Grenier[2], Mikhael Hadida[1], Julia Niemann[3], Lukas Raddatz[3], Kevin McHugh[4], Christophe Marquette[1], Magali Barbaroux[5], Emma Petiot[1]

[1]3d.FAB, Univ Lyon, Université Lyon1, CNRS, INSA, CPE-Lyon, ICBMS, UMR 5246, 43, Bd du 11 Novembre, Villeurbanne cedex, France
[2]CNRS, Inserm, CREATIS UMR 5220, U1206, Univ Lyon, INSA-Lyon, Université Claude Bernard Lyon 1, UJM-Saint Etienne, Lyon, France
[3]Sartorius Stedim Biotech Gmbh, August Spindler Strasse 11 Goettingen, D-37079 Germany
[4]Sartorius North America Inc.565 Johnson Avenue, Bohemia, NY 11716, USA
[5]Sartorius Stedim FMT SAS, avenue de jouques, 13400 Aubagne

elliott.cowles@univ-lyon1.fr

3d fab PRINTING FOR LIFE

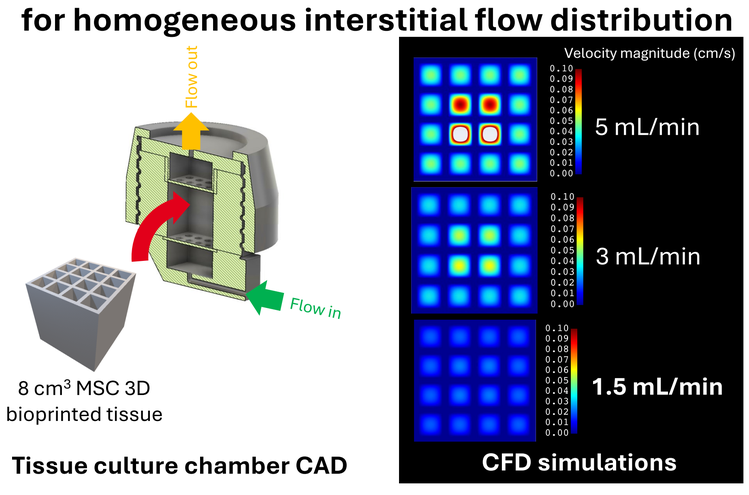
3D Innovation Lab

SARTORIUS

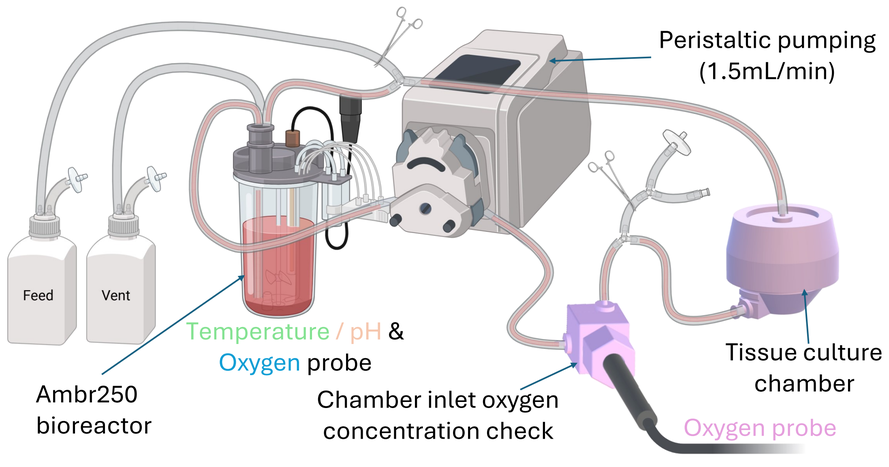
Given regulatory directives to reduce animal testing for pre-clinical trials and regenerative medicine applications, tissue engineering has emerged as a field with the goal to produce biofabricated tissues. Most current biofabricated constructs remain small (<1 $cm^3$) compared to physiological tissues, lacking complex architecture and associated functions of native tissues. Additionally, constructs are standardly cultivated in static plate with high dissolved oxygen (DO) levels and buffered pH, which is different to their *in vivo* local microenvironment. To study the effect of microenvironment on cell fate in 3D structures, control and analytical strategies need to be developed to determine boundary conditions and estimate local microenvironments in large constructs. Perfusion culture with precise physicochemical control, non-destructive monitoring and sampling could enable to do so, gaining understanding in spatial microenvironment characterisation within tissue culture processes. The latter strategy is presented herein using a large porous 8$cm^3$ mesenchymal stem cell (MSC) bioprinted tissue model.

## 1. PHYSICO-CHEMICAL ENVIRONMENT CONTROL

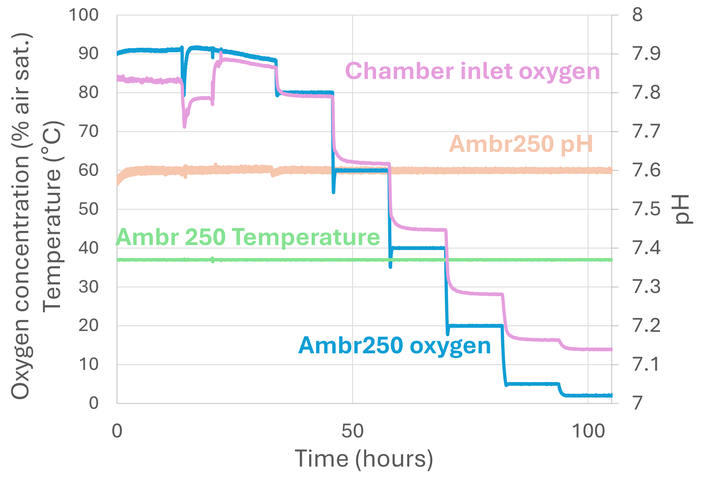
### Design of tissue culture chamber for homogeneous interstitial flow distribution




### Regulated perfusion setup

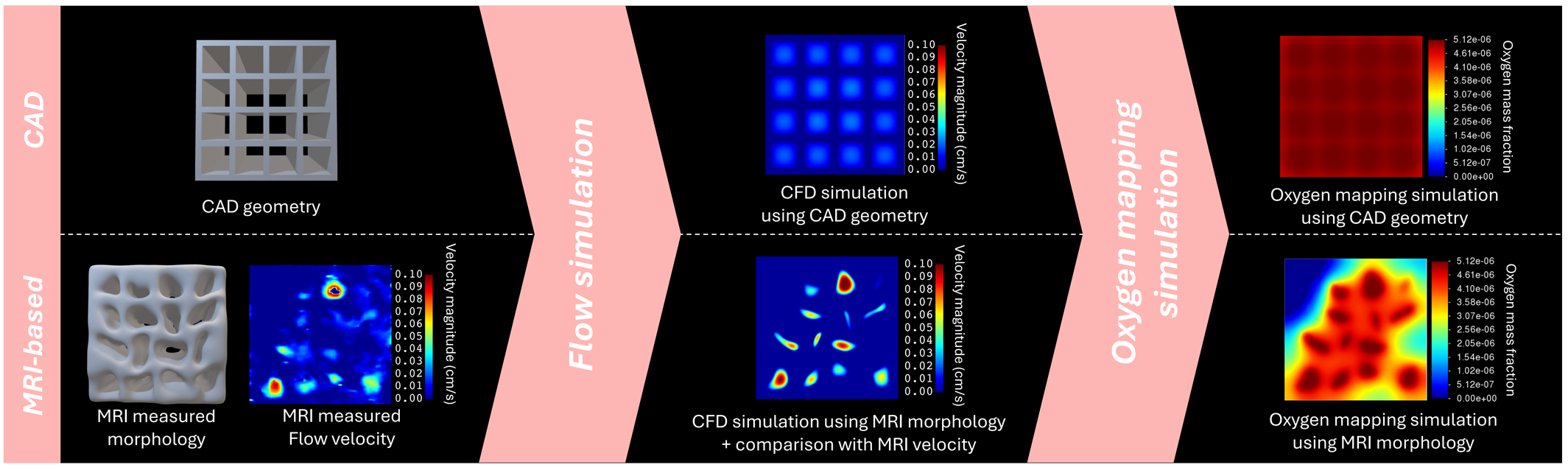



### Physicochemical Parameter Regulation




- **Design of a culture chamber for homogeneous large 8$cm^3$ tissue perfusion for 21 days (1.5mL/min).**
- **Establishment of control loops for temperature, pH and DO regulation covering a wide oxygen concentration range (20%-80% air saturation).**
- **Setpoint-dependent DO offset identified between measurements in Ambr250 and chamber inlet are linked to tubing permeability to oxygen.**

## 2. MICROENVIRONMENT CHARACTERISATION AND FLOW MODELLING VALIDATION

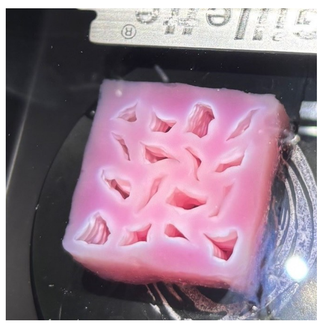



| Simulation parameters | Value |
|---|---|
| Oxygen diffusivity | 3x10$^{-9}$$m^2$/s |
| Tissue porosity | 50% |
| Oxygen volume consumption | 4.62x10$^{-3}$ kg/($m^3$.s) |
| Oxygen inlet concentration | 5.12 x10 mg/L |

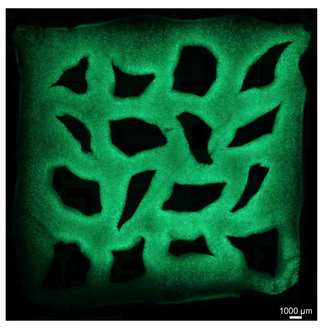
- **Morphological differences can be identified between expected (CAD) and real (MRI) bioprinted tissue internal architecture.**
- **MRI Velocimetry analysis demonstrates heterogeneous flow distribution compared to expected homogeneous distribution.**
- **Predicted microenvironment using literature and calculated parameters show discrepancies with expected CAD-based microenvironment.**

## 3. MESENCHYMAL STEM CELL LIVE CELL DISTRIBUTION IN RESPONSE TO MICROENVIRONMENT

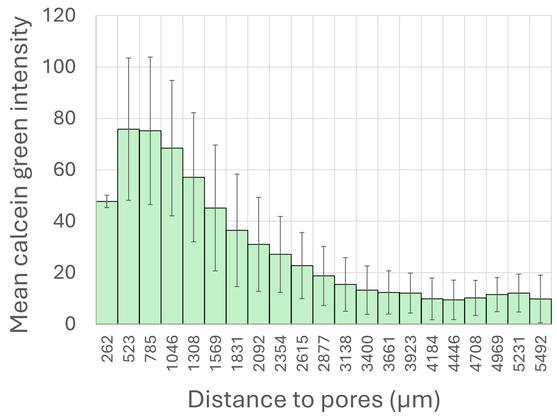
### 3D cell response characterisation



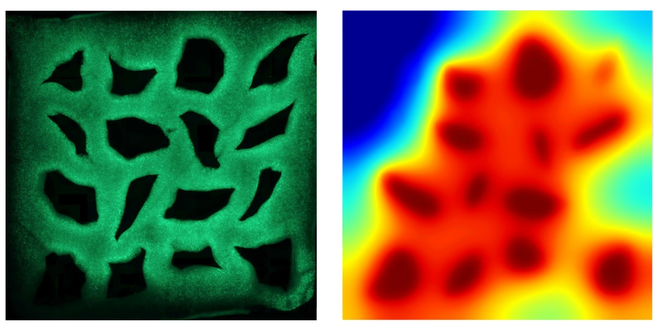
1mm-thick slices of MSC bioprinted tissue (vibratome)



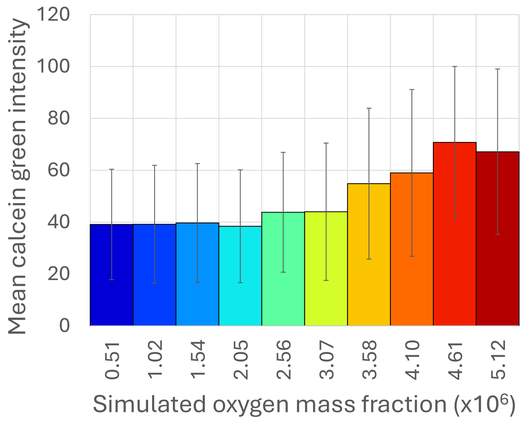

Live cell distribution (calcein green staining)




### 3D cell response/microenvironment correlation



Oxygen mapping and live cell distribution alignment




- **Tissue slicing and characterisation allow to identify local cellularisation gradients in the 3D structure.**
- **Data shows more live cells calcein green signal closer to pores, linked to more live cell activity and diffusion-related gradients.**
- **Preliminary simulations shows higher oxygen concentrations may be associated to slightly higher live cell activity.**

## TAKE HOME MESSAGE

- **A novel method for large tissue culture in controlled environments using perfusion, probes and associated regulations has been developed.**
- **Environment prediction using CAD-based CFD simulations is insufficient to describe complex geometries and requires real morphology characterisation.**
- **A more representative model combining nondestructive MRI and CFD simulations allows to estimate spatial physicochemical environment and evaluate their impact on 3D cell response.**
- **Additional data using different inlet concentrations and hydrogel/cell specific simulation parameters would allow to gain in robustness and correlate 3D cell response with selected microenvironments.**

UNIVERSITÉ DE LYON | Université Claude Bernard Lyon 1 | cnrs | ICBMS LYON