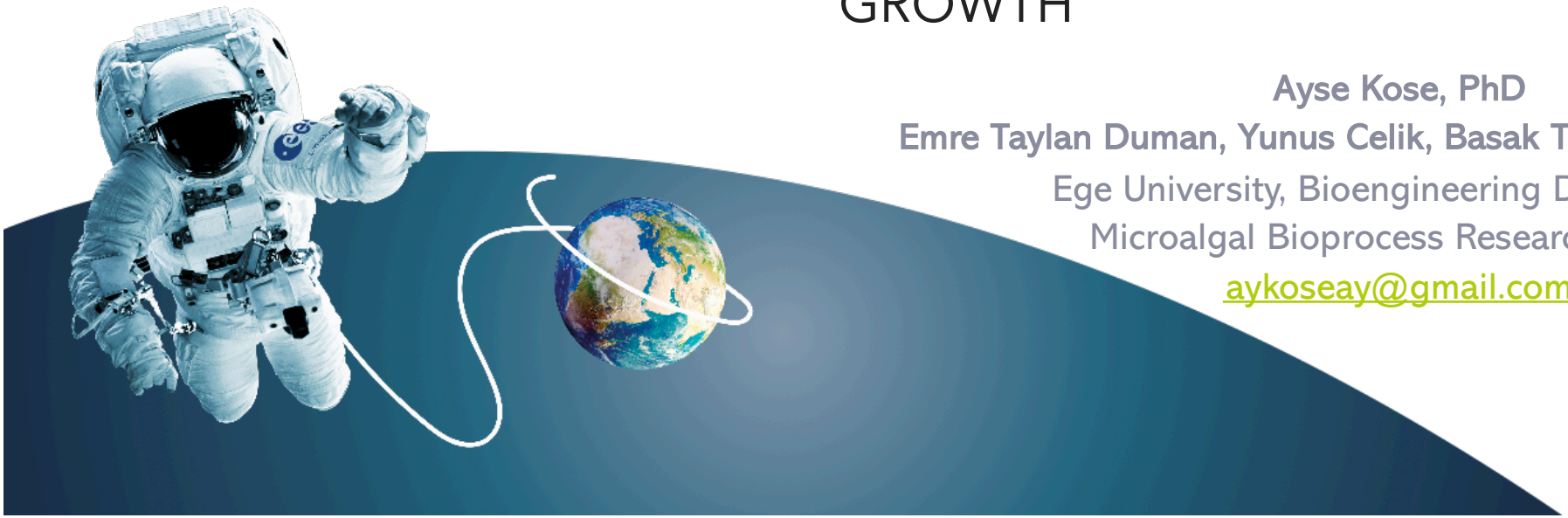
MELiSSA
MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

CREATING A CIRCULAR FUTURE

MICROGRAVITY MIMETICS ON THE DEVELOPMENT OF MULTIFUNCTIONAL BIOREACTORS SYSTEMS FOR EFFICIENT CELL GROWTH



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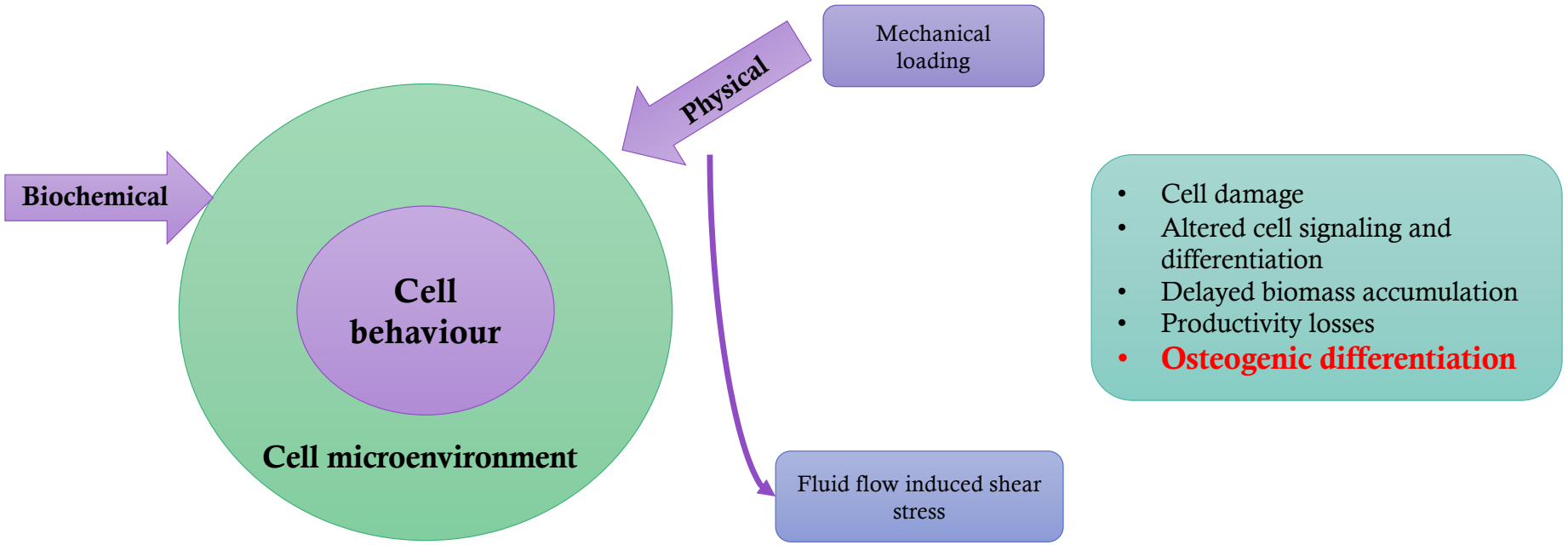
OUTLINE

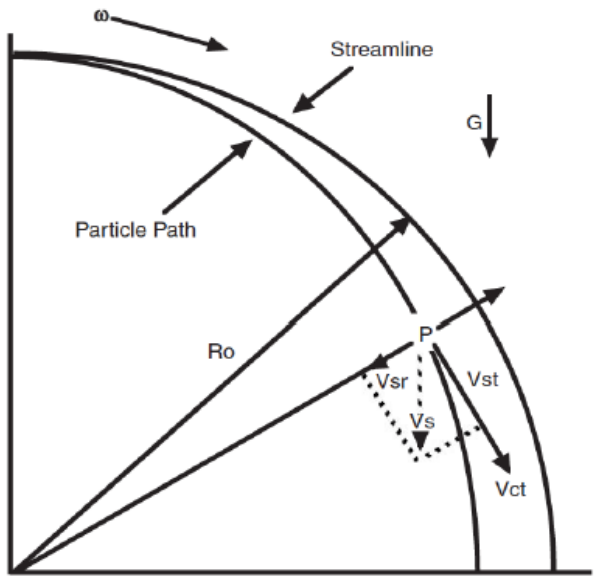
- Shear stress and cell response
- Microgravity biomimetics
- 3D cell cultures and bioreactors
- A novel bioreactor with low shear stress
- Case study 1: Cell Wall deficient *Chlamydomonas reinhardtii*
- Case study 2: HepG2 spheroids for drug/biomolecule testing

SHEAR STRESS



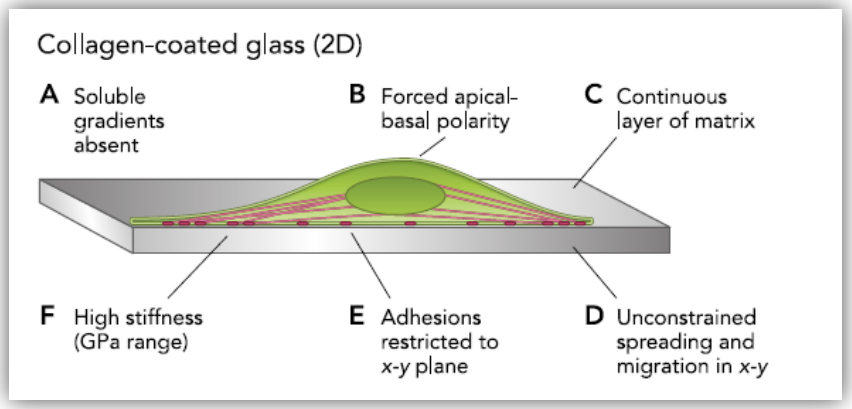
PRINCIPLE OF THE MECHANISM: MICROGRAVITY MIMETICS

- The forces acting on a particle (P) rotating in a fluid are shown. Gravity-induced sedimentation (Vs) can be resolved into radial (Vsr) and tangential components (Vst and Coriolis, Vct). There is an outwardly directed vector due to centrifugal force.

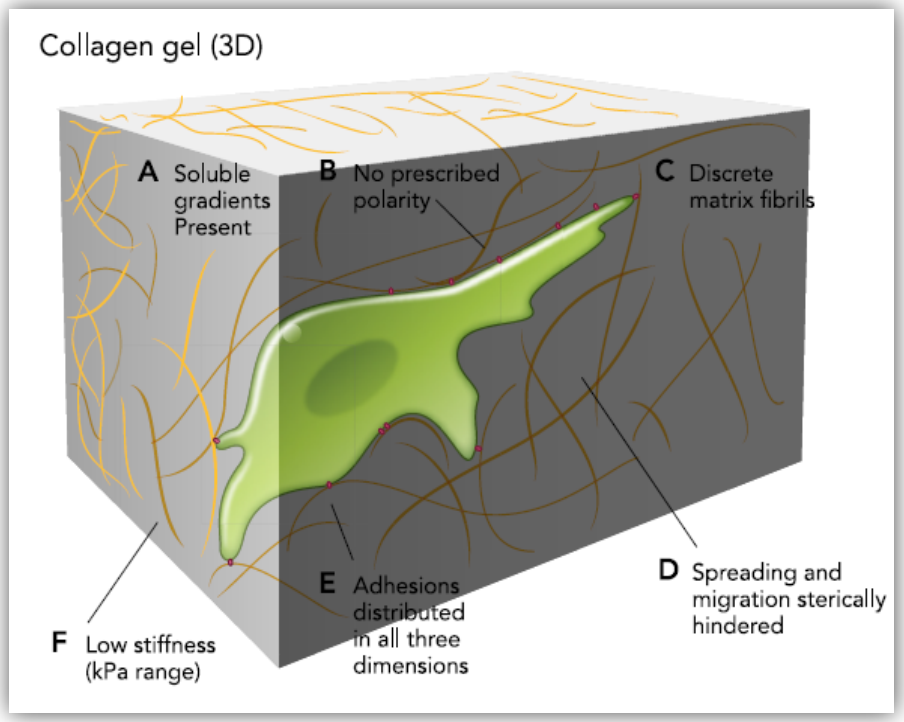


https://synthecon.com/files/248_whitepaperoriginal.pdf

WHY 3D CELL CULTURE INSTEAD OF 2D?



Cells in 2D and 3D microenvironments interact differently with their surroundings due to differences in the cues, mechanical and chemical, that they experience.



Baker B.M. ve Chen C.S., 2012

3D CELL PROLIFERATION IN BIOPROCESSES

STATE OF THE ART

Types of bioprocesses	Advantages	Disadvantages
Lab Scale (T flasks, spinner flasks, roller bottles, hollow fiber bioreactors)	• Easy to management • Parallel scale up	Using many units causes time loss, there is no continuous monitoring of process conditions (without monitor)
Bach or Fed-Bach Bioreactors	• Homogeneous production • Easy to scale up • Flexible bioprocess possibilities	Towards the end of the process, vitality % decreases, toxic metabolites may accumulate
Perfusion Bioreactors	• High cell density • Easy to manage culture conditions • Real steady-state is possible • Excellent mechanical loading for bone tissue engineering applications	The validation process is long and complex, it is designed for a special product, so system is not flexible

REQUIREMENT

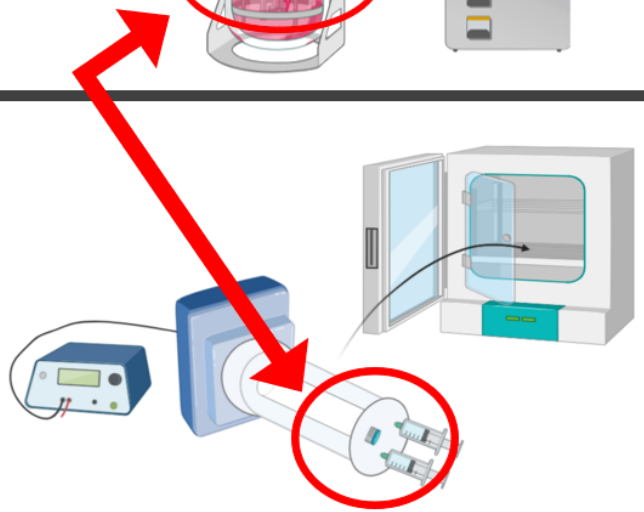
- Bridging the lab scale with industrial models
- Creating flexible bioprocess operation modalities
- Realistic approaches in tissue culture applications
- Industrial acceptability of micro-3D cell proliferation systems

BUT…. HOW

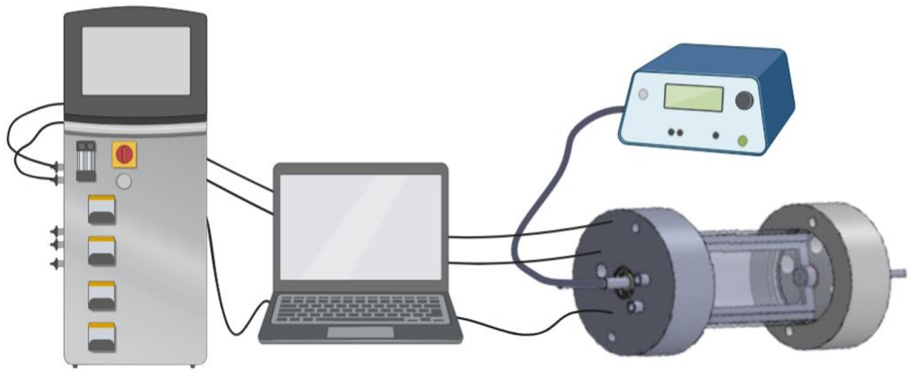
WE NEED A NEW DESIGN

CSTR

RCCS



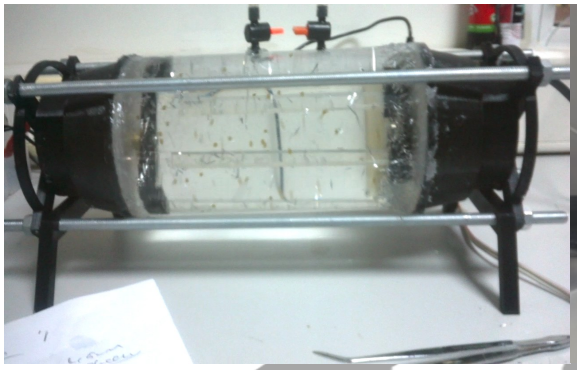
LSB-R

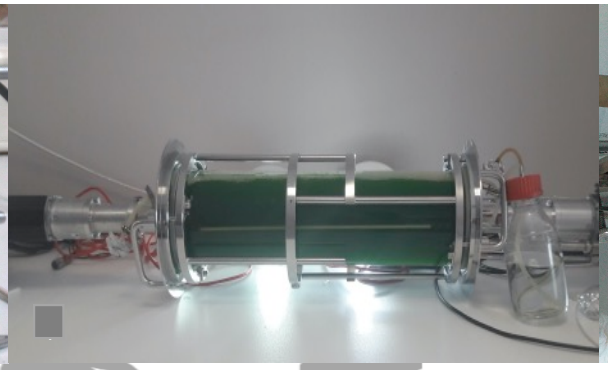


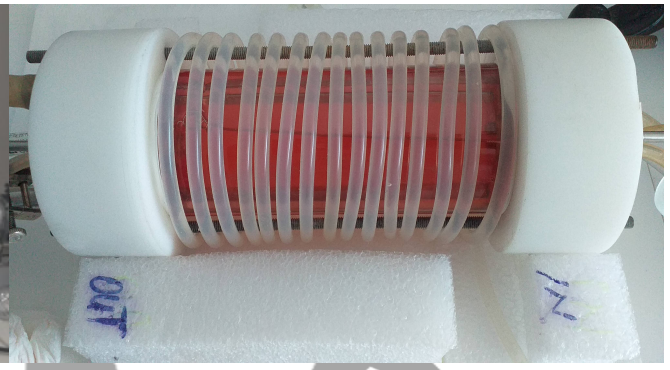
Patent Pending: Turkish Patent Institute (TPE) Patent Number: 2015/14967

LSBR

LSBR EVOLUTION







?

- 500 mL
- Magnetic stirred
- 3D Printer / Plexi
- Mixing analysis

- 5L
- Mechanically stirred
- Glass
- Microalgal cultivation
- CFD analysis

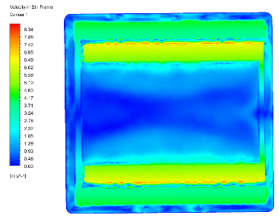
- 500 mL
- Mechanically stirred
- Glass / POM
- Animal cell production
- CFD analysis

STEP BY STEP LSB-R DESIGN

IDEA

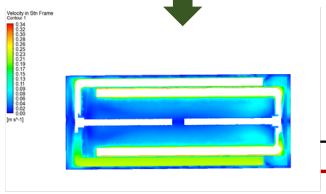
Design
Prototyping
(500 ml)

CFD Simulations



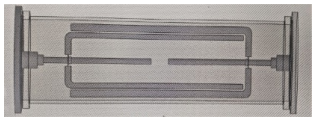
Design
Prototyping
(5 L)

CFD Simulations



DESIGN

Final Construction



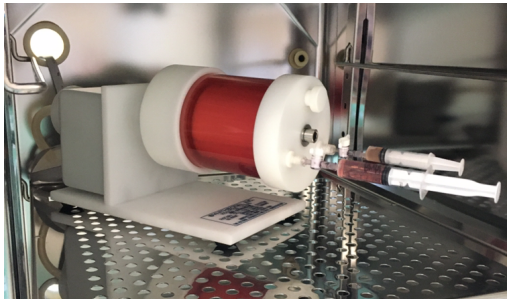
CFD Simulations

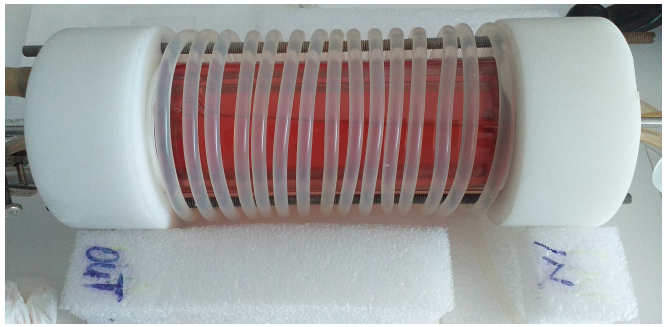
LSB-R

CSTR

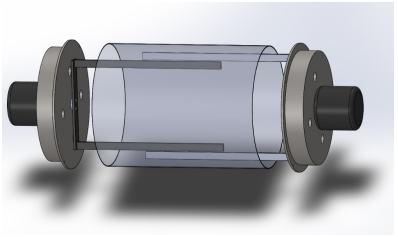
RCCS

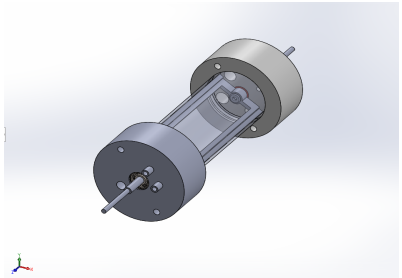
OPERATION

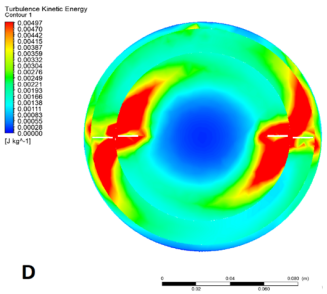


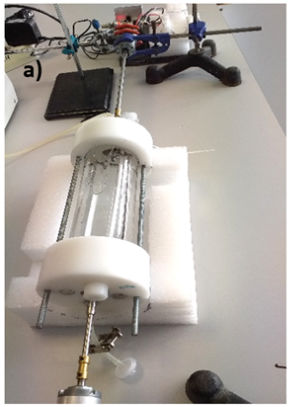


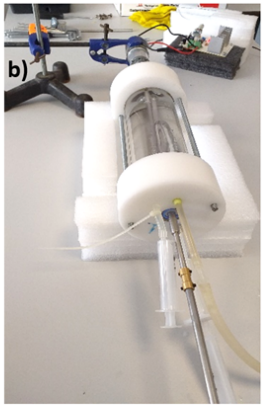
FROM FANTASY TO REALITY





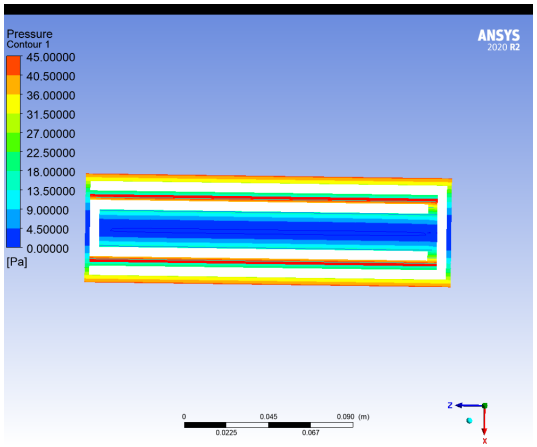


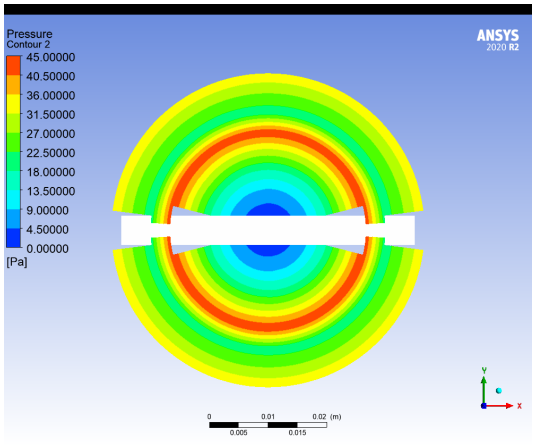




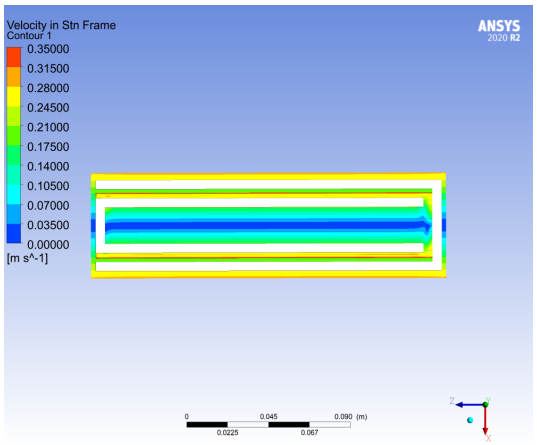
HOW IS THE FLOW IN THE LSBR?

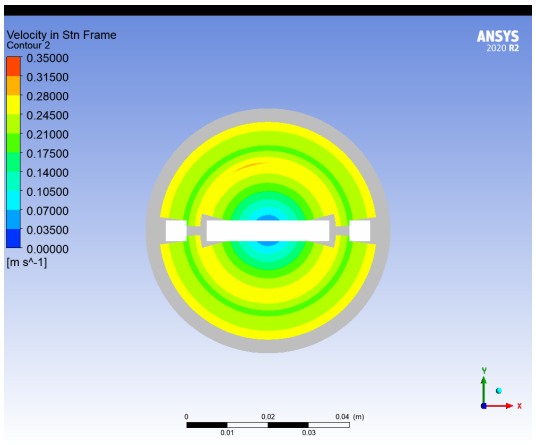
Pressure Gradient





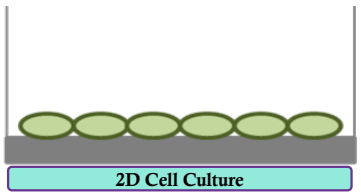
Velocity Gradient



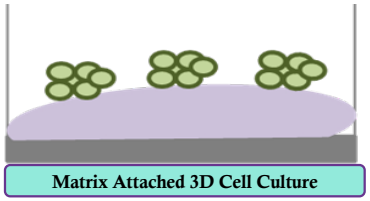


LSBR VS. EXISTING SYSTEMS

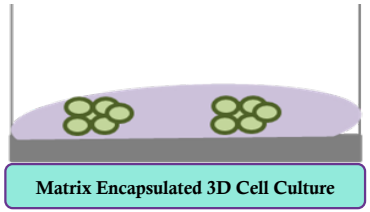
Systems	Shear-stress	Mass Transfer	Dimensionality	Co-location of dissimilar cells	Sensors and online control
RCCS	Very low	Excellent	Excellent 3D	Excellent	None
LSBR	**Very low**	**Excellent**	**Excellent 3D**	**Excellent**	**Excellent**
T-Flasks	None	Adequate 2D	3-4 layers 2D	Limited	None
Static 3D (Matrix-gels)	None	Limited 3D	Limited	Limited	None
Stirred suspension cultures	Medium/ High	Good	Very limited 3D	Very limited	Excellent
Hanging drop	None	Limited 3D	Limited 3D	Limited	None
Roller Bottles	Medium	Good 2D	3-4 layers 2D	Very limited	Excellent



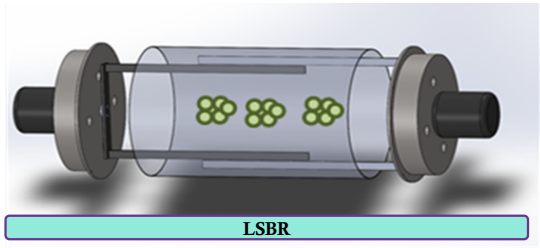
2D Cell Culture



Matrix Attached 3D Cell Culture



Matrix Encapsulated 3D Cell Culture



LSBR

CASE STUDY1: PRODUCTION OF CELL WALL DEFICIENT *CHLAMYDOMONAS REINHARDTII* STRAINS

- Shear sensitive strains:
  - **CC-2853** cell wall deficient/motility impaired
  - **CC-3491** as wall deficient/motile

❑ **Mixing time:** 23±2 sec

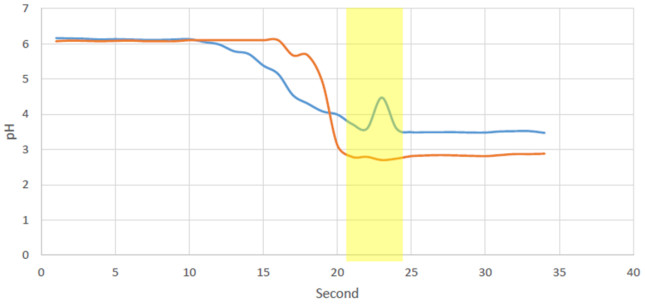
❑ **Surface/Volume ratio:** 0.55±0.01

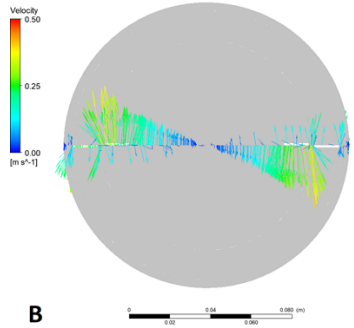
❑ **Average shear stress:** 0.8 Pa

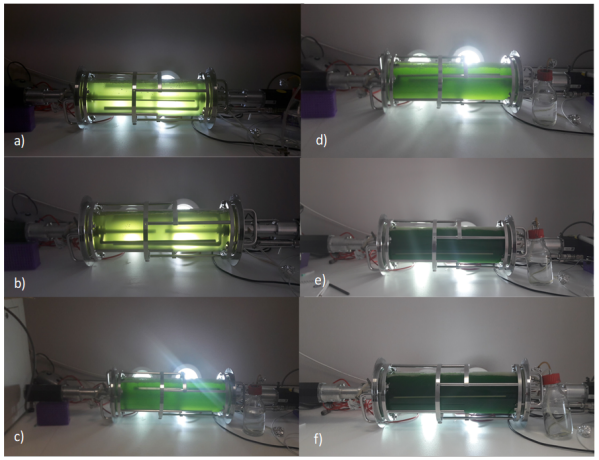
❑ **Outer and inner blade speed:** 60 rpm, 35 rpm

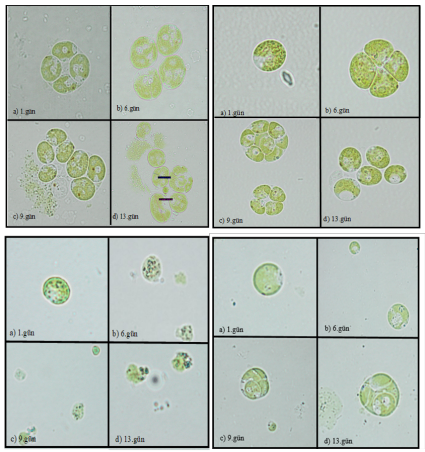
❑ **Reynolds number:** 9950

❑ **Specific growth rate:**
  - C-3491-0.38±0.07 day^{-1}
  - CC-2853- 0.29±0.02 day^{-1}



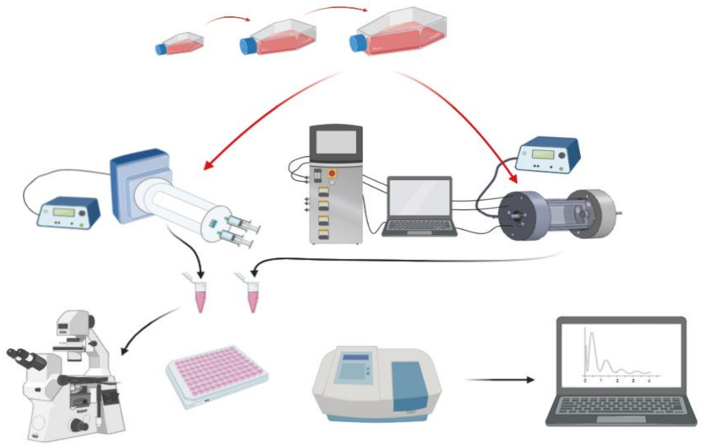


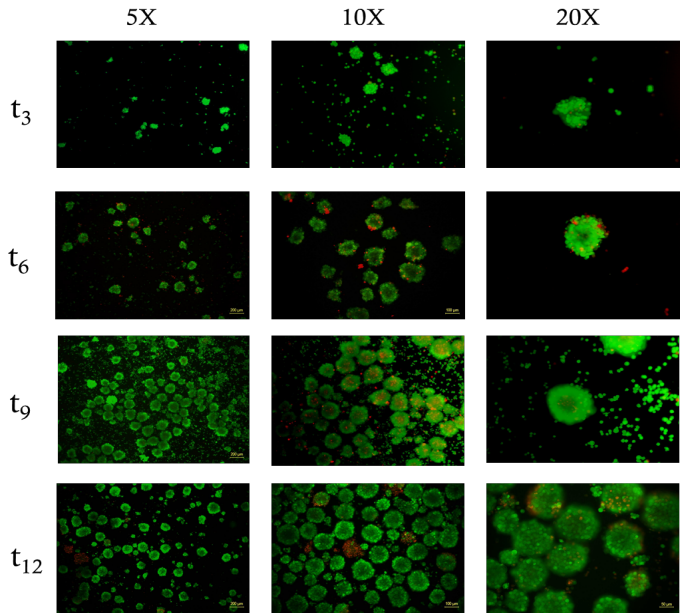




CASE STUDY 2: HEPG2 SPHEROIDS PRODUCTION FOR DRUG TESTING

- ❑ TUBITAK Research Project
- ❑ CFD analysis of LSBR
- ❑ Model validation in 500 ml LSBR
- ❑ Determination of shear stress and hydrodynamic forces
- ❑ Cell proliferation and spheroid formation of HepG2 cells
- ❑ Comparison of 2D and 3D cell growth
- ❑ Model toxicity experiments
- ❑ Measurement of liver-like responses on ethanol toxicity with HepG2 spheroids





MEET THE TEAM

Assoc. Prof. Suphi Ş. ÖNCEL

- Bioprocess engineering
- Bioreactor design
- Biohydrogen
- Microbial fuel cells
- Clean energy engineering
- Microalgal bioprocesses

Res. Assist. Ayşe KÖSE, PhD

- Bioprocess engineering
- Bioreactor design
- Peptides
- Natural molecules
- Tissue engineering

Emre Taylan DUMAN, PhD Student

- Computational design
- CFD analysis
- Bioinformatics

Başak TUĞCU, Msc Student

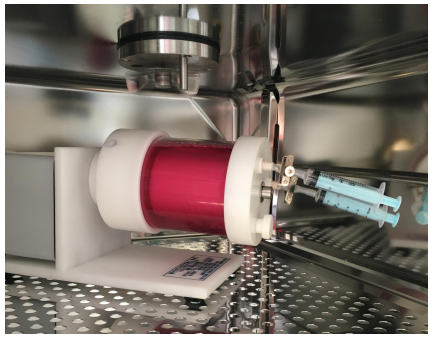
- Cell& Tissue engineering
- Spheroids applications

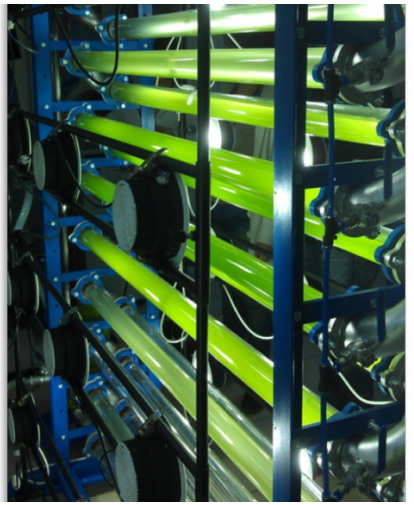
Yunus ÇELİK MSc Stdent

- Bioprocess engineering
- Bioreactor design
- CFD analysis

RESEARCH FOCUS

- Bioreactor design
- Bioprocess development
- Integration of process analytic technologies to bioprocesses (Bio-PAT)
- Development of marine nanobioprocesses
- Tyrosinase inhibitors from (microalgal) peptides
- Microbial fuel cell development
- Photobioreactor development –design and operation
- 3D cell growth on microgravity-like environment
- Biohydrogen production
- Algal cosmetics
- Vaccine production





MELISSA

MICRO-ECOLOGICAL
LIFE SUPPORT SYSTEM
ALTERNATIVE

TÜBİTAK

EGE ÜNİVERSİTESİ 1955

ege biyomühendislik

EGE ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ 1982

THANK YOU.

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PARTNERS

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