Applications of Bioreactors in Tissue Engineering

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NIH/NIBIB Center for Engineering Complex Tissues (CECT)

3rd Annual 3D Printing and Biofabrication Workshop

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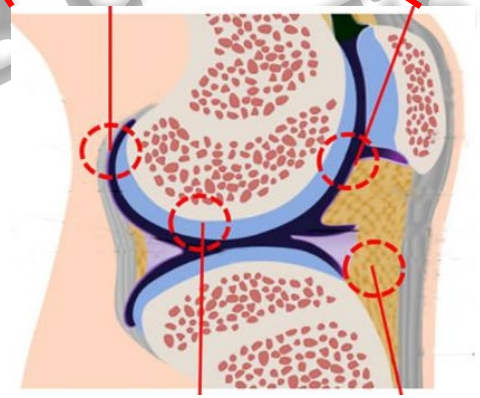
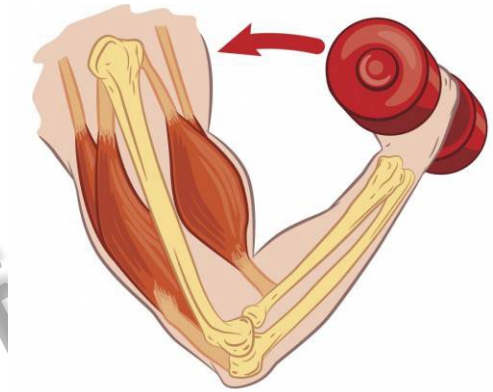
CECT Center for Engineering Complex Tissues

The Human Body: A Dynamic Bioengineering Problem

Running



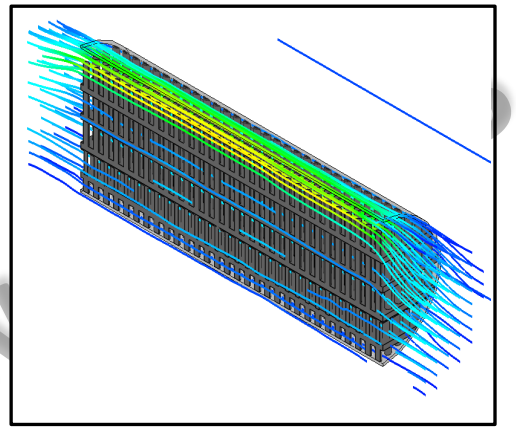


Muscle movements

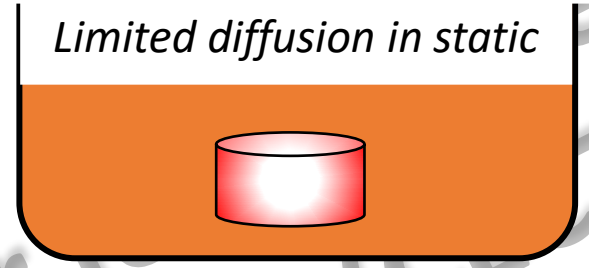
Blood flow

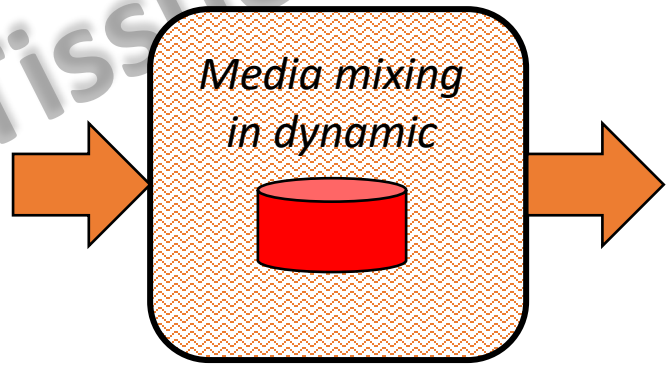
Role of Bioreactors in Tissue Engineering

Biomimicking the physiological dynamics of our body for more reliable studies



Biomimicry





Enhanced nutrient diffusion

Biomanufacturing and Scale up

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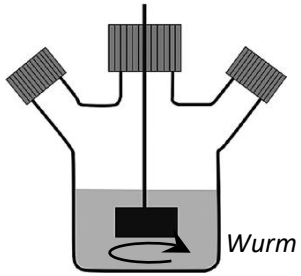
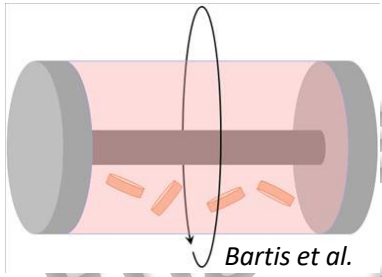
Traditional Bioreactors

Spinner Flask



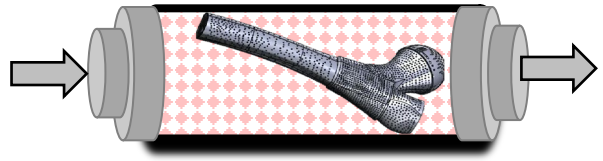
Wurm et al.

Rotating Wall



Bartis et al.

Perfusion Bioreactors



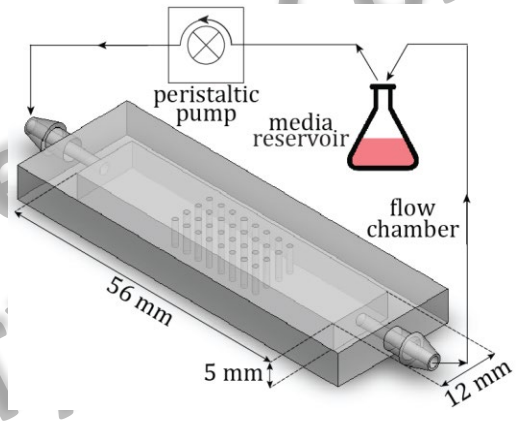
Pros	• Easy to use and maintain • Culture cell suspensions and scaffolds	• Easy to use and maintain • Can generate microgravity conditions	• Can do continuous process • Internal and external mixing • Controlled shear forces
Cons	• Difficult to use in mass production • Complex fluid mechanics • Only external mixing	• Not feasible for large scaffolds • Only external mixing • Potential scaffold damage	• Difficult to maintain sterility • Several moving parts • Non-trivial sample handling

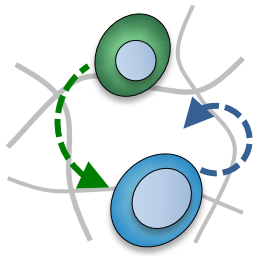
Maria Joao De Jesus Florian Maria Wurm, "Medium and Process Optimization for High Yield, High Density Suspension Cultures: From Low Throughput Spinner Flasks to High Throughput Millilitre Reactors", Bioprocess International 2009

Bartis et al, Three dimensional tissue cultures and tissue engineering, University of Pécs, 2011

Need for Tissue-Specific Bioreactors

- Engineering design for tissue specificity
  - Geometry
  - Size
  - Shape
  - Mechanical stimuli





Tissue	Application	Tissue	Application
Bone	• Shear stress • Mechanical stress	Blood vessels	• High vs low pressure flow
Cartilage	• Compression • Shear stress	Skin	• Stratified nutrient exchange
Tendon/Ligament	• Tension • Rotation	Barriers (e.g. blood-brain; placenta)	• Media isolation vs. controlled permeation
Heart	• Electrical stimulation • Pulsatile	Large scaffolds	• Efficient nutrient exchange • Efficient vascularization

3D Printing for Bioreactor Design

Customization and rapid assessment of *in vitro* tissue design

3D Printers

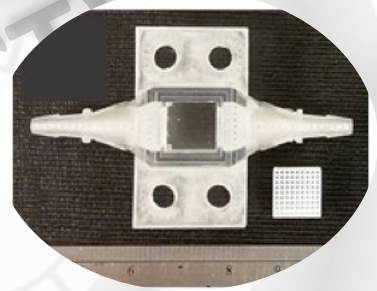
Bioplotter

Perfactory

6-axis BioBot

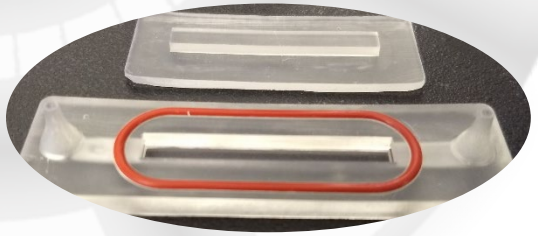
Enabling Technologies

Large volume bioreactors for tissue maturation



Dual-chambered bioreactors for dynamic multi-tissue engineering

Microfluidic bioreactors for mixing and bead generation



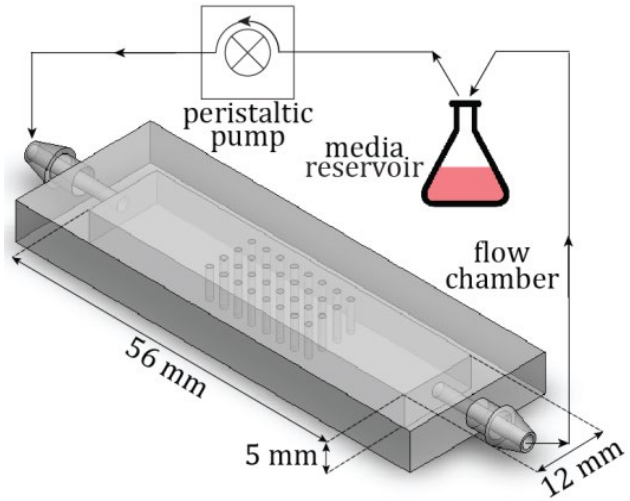
Microphysiological bioreactors with controlled internal environments

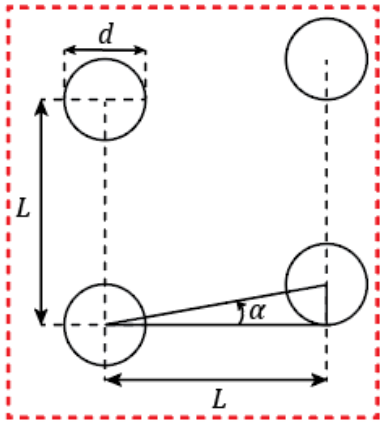
Selected References
Biotechnology & Bioengineering 116, 181-192 (2019)
Biomaterials 185, 219-231 (2018)
Tissue Engineering Part A 24, 1715-1732 (2018)
Biomacromolecules 18, 3802-3811 (2017)
Advanced Healthcare Materials, 319-325 (2016)
Advanced Materials 27, 138-144 (2015)

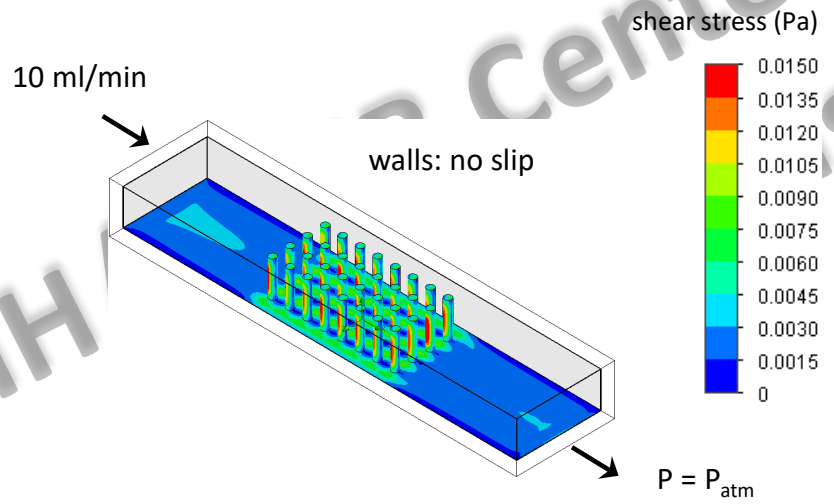
John P. Fisher
Fischell Family Distinguished Professor & Department Chair
Fischell Department of Bioengineering, University of Maryland

ECT Center for Engineering Complex Tissues

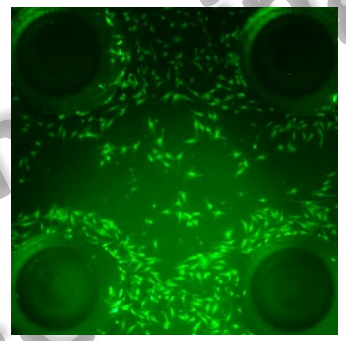
Microphysiological BRs: Geometry control





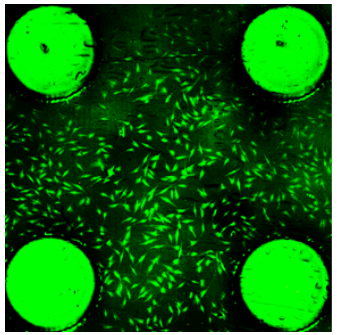


Cell Localization

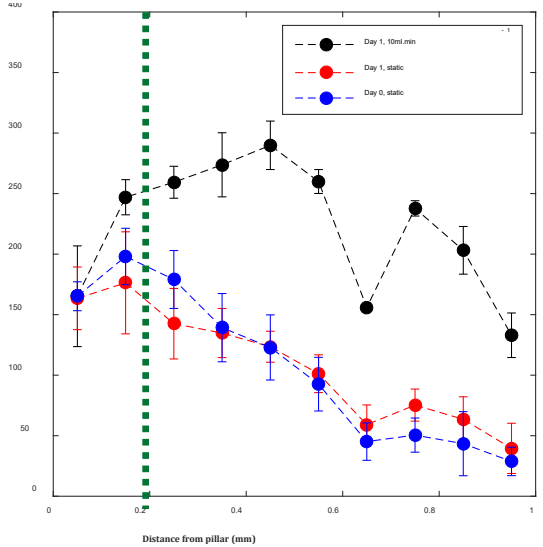


hMSC localization near pillars

Flow



Uniform distribution



Lembong et al., Tissue Eng Part A. 2018 Dec;24(23-24):1715-1732.

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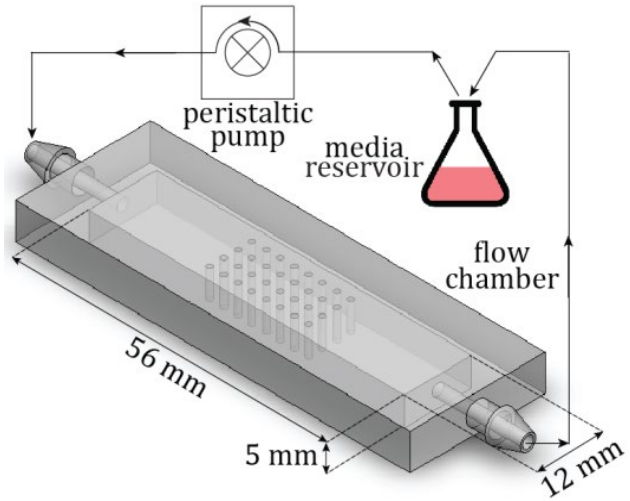
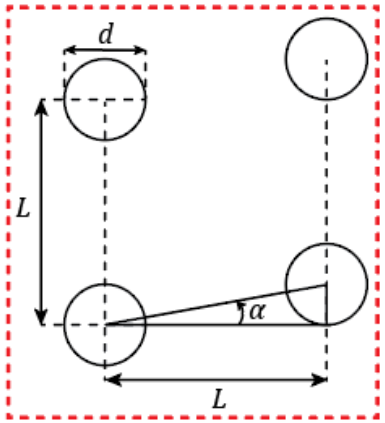
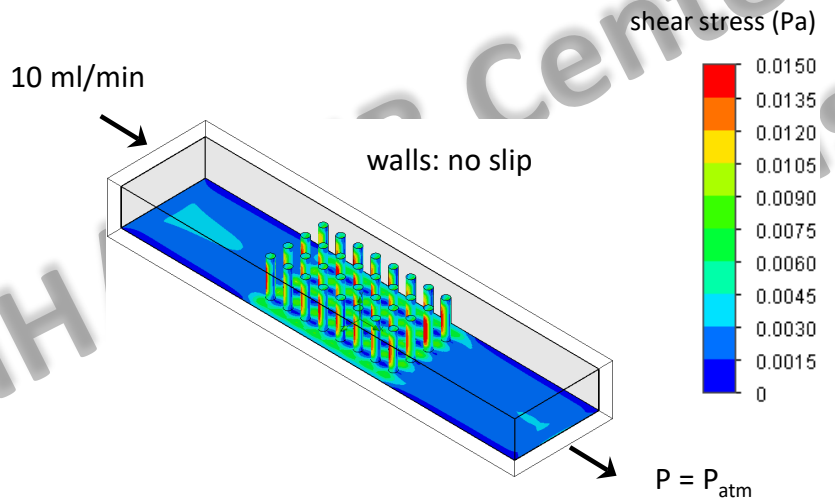
Microphysiological BRs: Geometry control

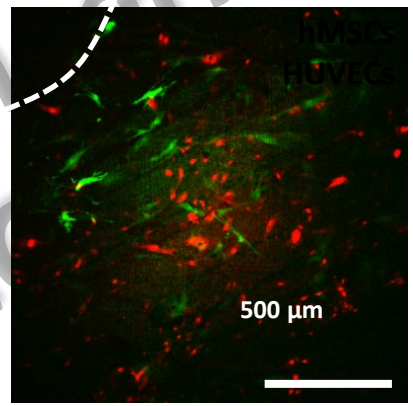


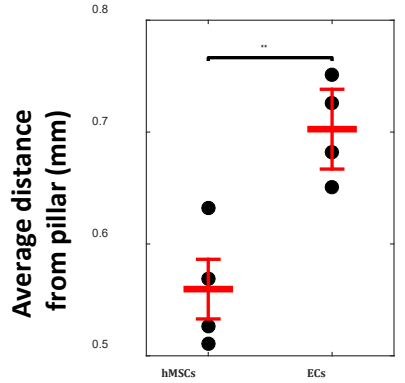




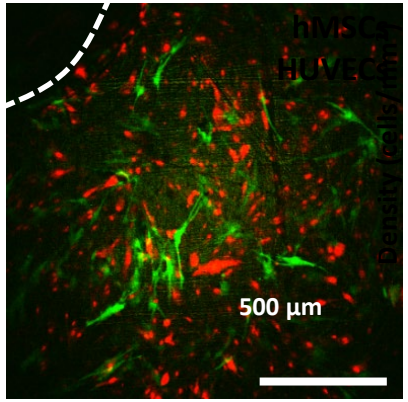
Cell Patterning

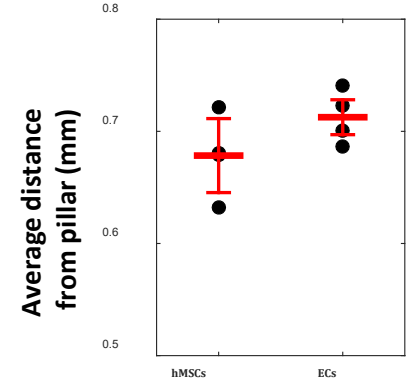
Seeding order: hMSCs, then HUVECs





Seeding order: HUVECs, then hMSCs





Lembong et al., Tissue Eng Part A. 2018 Dec;24(23-24):1715-1732.

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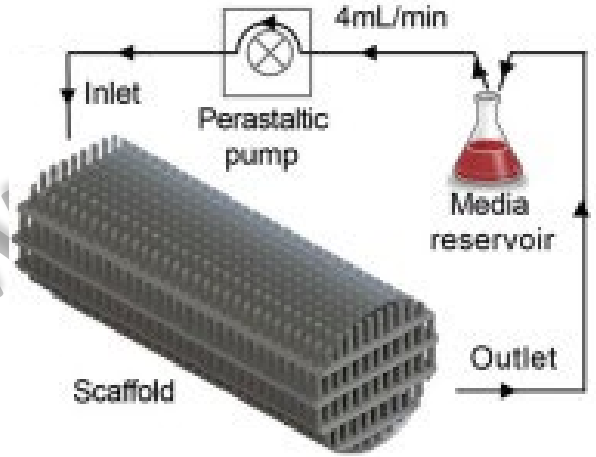
Engineering Large Surface Area to Volume Ratios

Significant reduction in operational unit volume

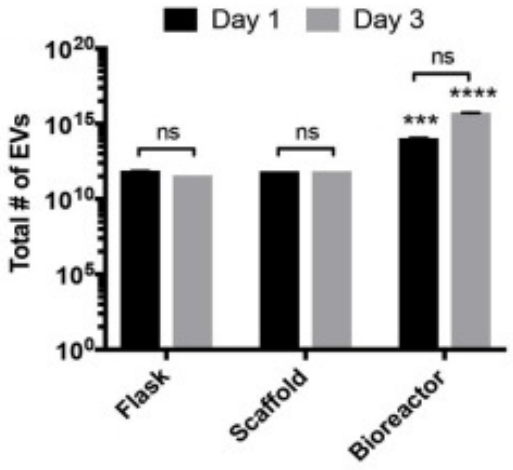
T75 flask ***(~ 338 cm³)***

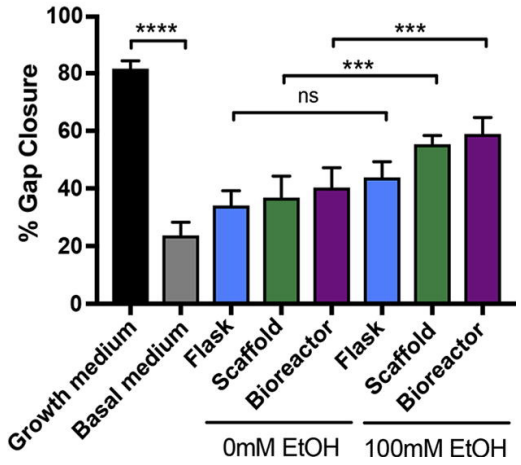
3D printed bioreactor
(~ 2.5 cm³)



- Extracellular vesicles (EVs) as therapeutic vectors
- Address EV scalability using a bioreactor system approach via 3D-printed scaffolds

Significant increase in EV production with bioreactor



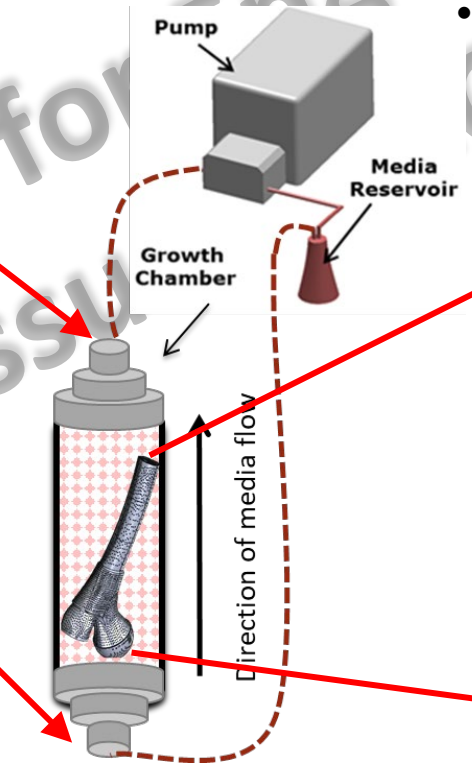


Patel et al., *Acta Biomaterialia*, 95: 236-244

Bioreactors for Large Scaffolds

Scale up using the Tubular Perfusion System (TPS) bioreactor

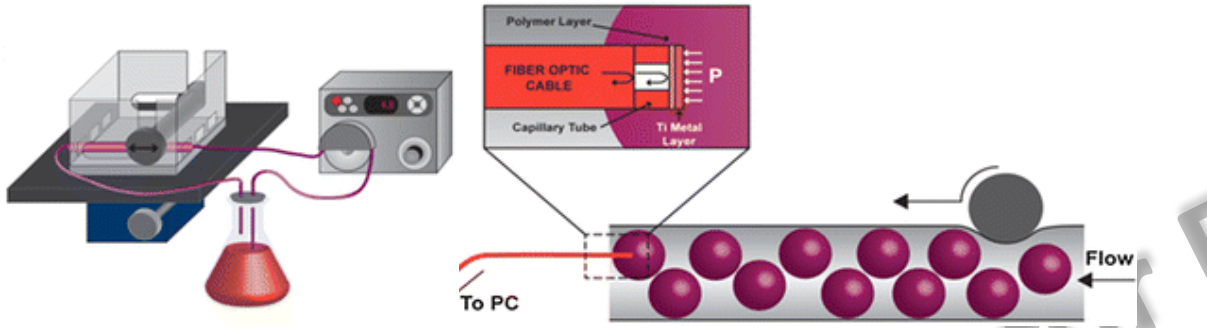
- Compressing a 20,000 cm^2 culture area into a 2800 cm^3 volume for hMSC culture
- Top-half of an adult human femur (200 cm^3)
- 20-fold increase over previously reported volumes

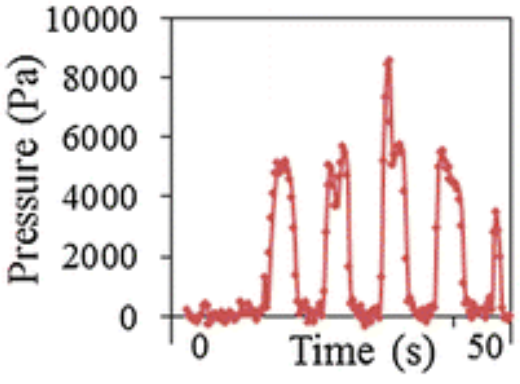


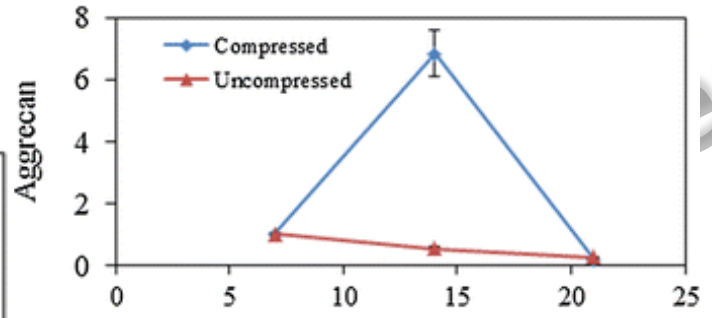
Nguyen et al. Tissue Engineering: Part A, Vol. 22, No. 3, 2016

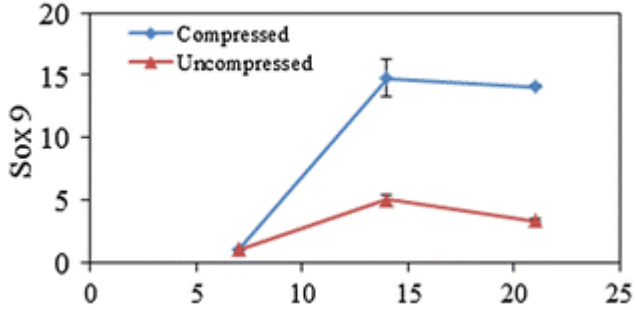
Bioreactors for Mechanical Stress

Compression



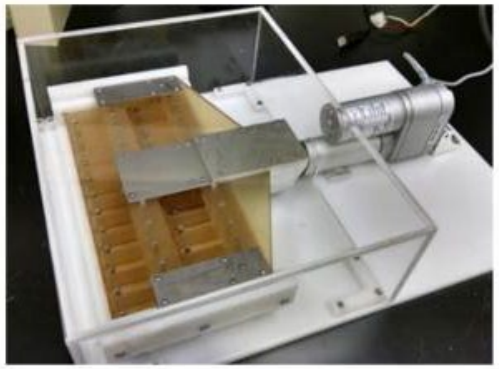


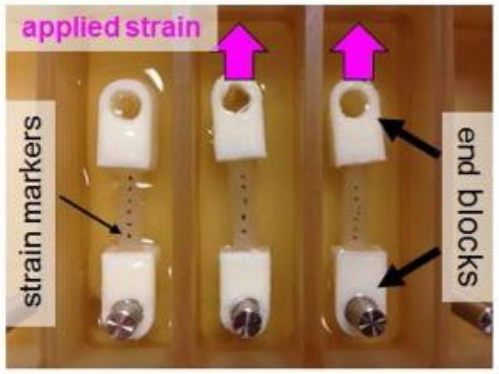


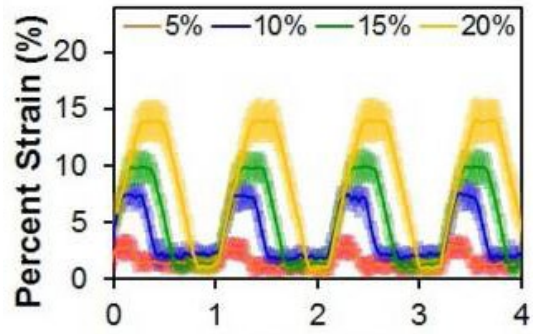


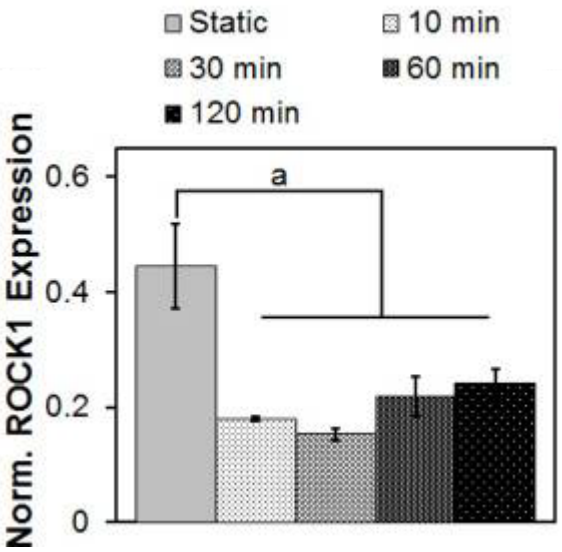
Guo et al., Ann Biomed Eng. 2016 Jul;44(7):2103-13

Tension



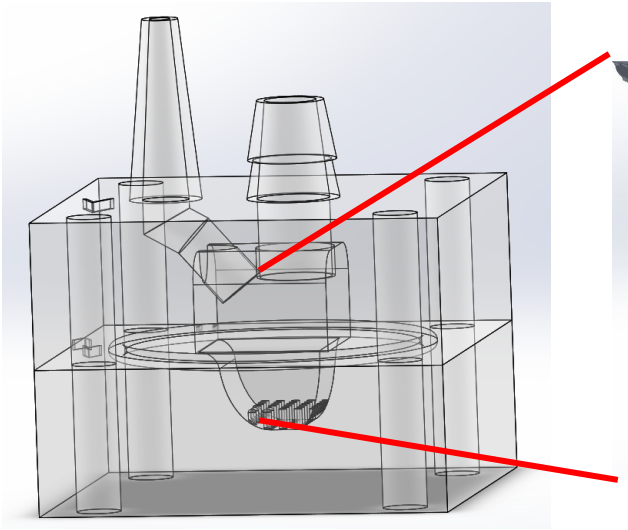




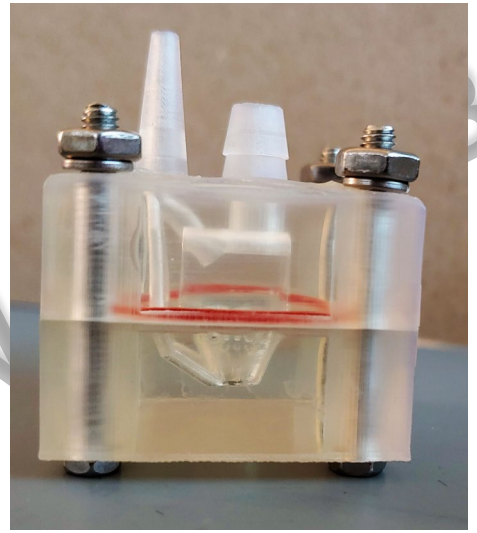


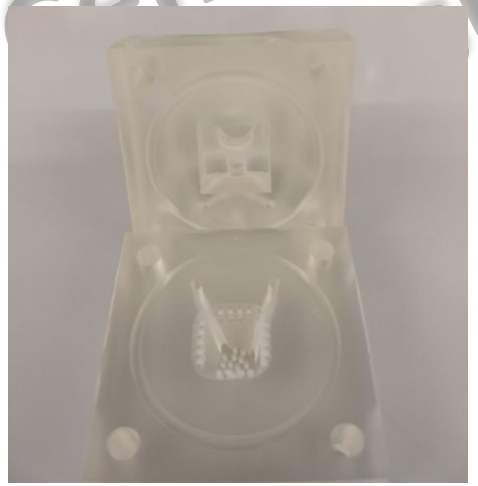
Grier et al., 2017 Mar 20;33:227-239

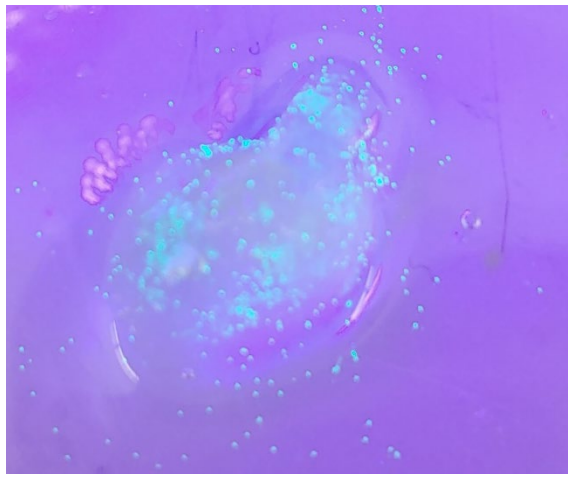
Chambered Bioreactors for Tissue/Organ Culture



- Dr. Brenda Ogle (U. Minnesota)
- Bioreactor to allow fluid flow through human chambered muscle pump (hChaMP)
- Maintain physiological flow rates (10-20 mL/min)
- Allow for assessment of hChaMP
  - Easy removal of hChaMP
  - Assessment in bioreactor

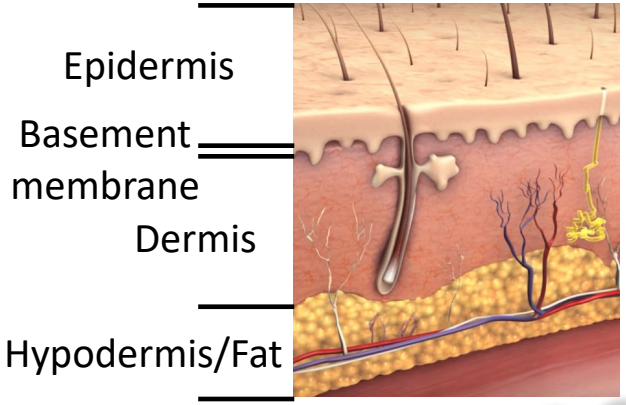


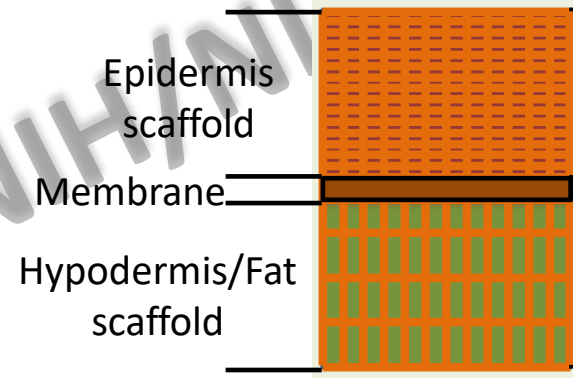


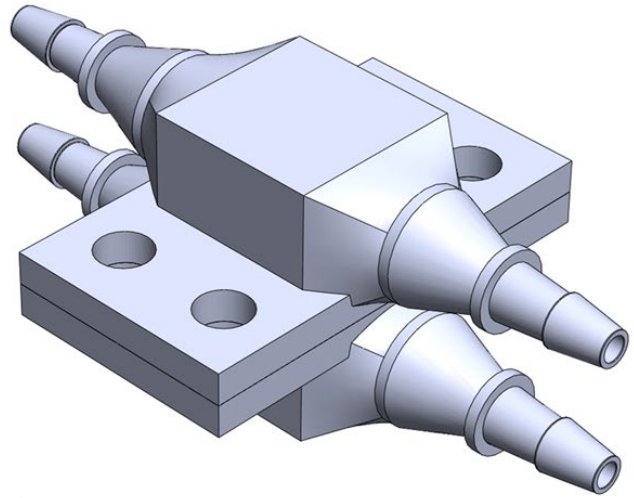


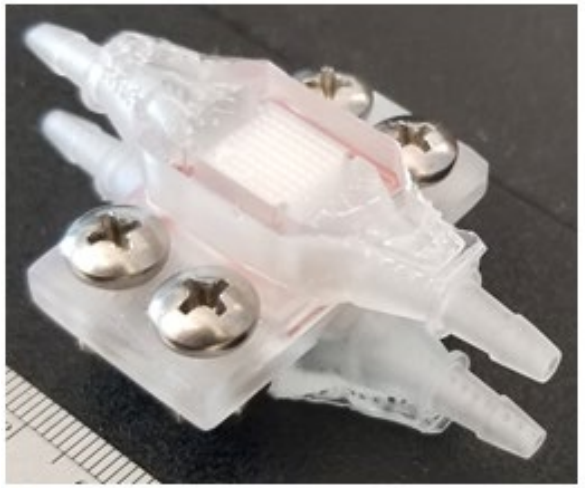
Dual Chambered Bioreactors for Stratified Cultures

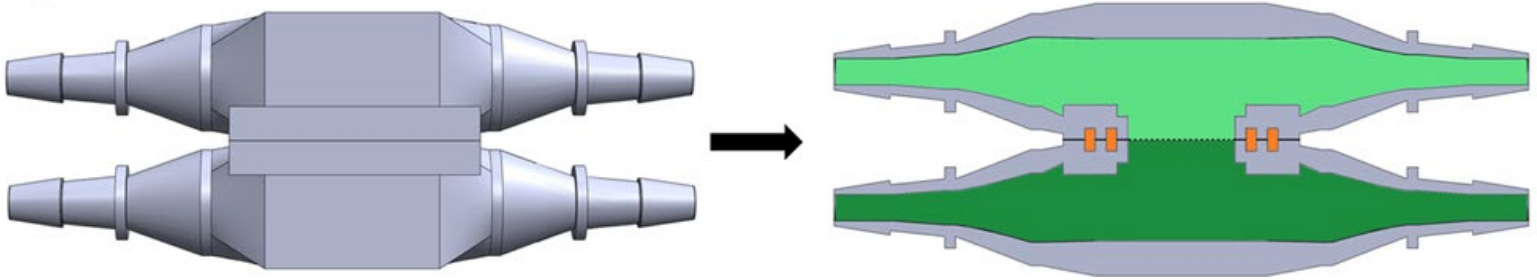
Skin Tissue Engineering











Navarro et al., Biotechnology and Bioengineering. 2019;116:3253–3268

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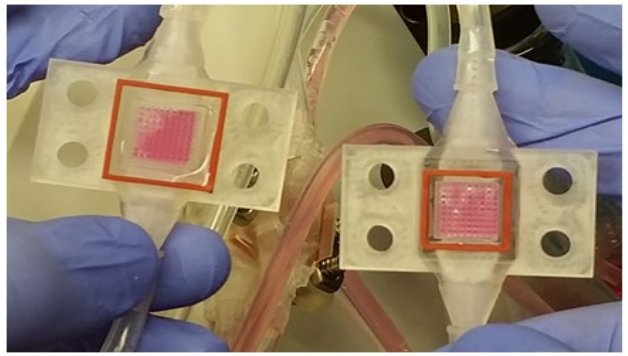
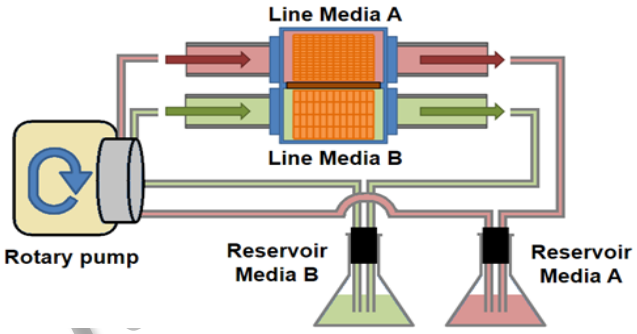
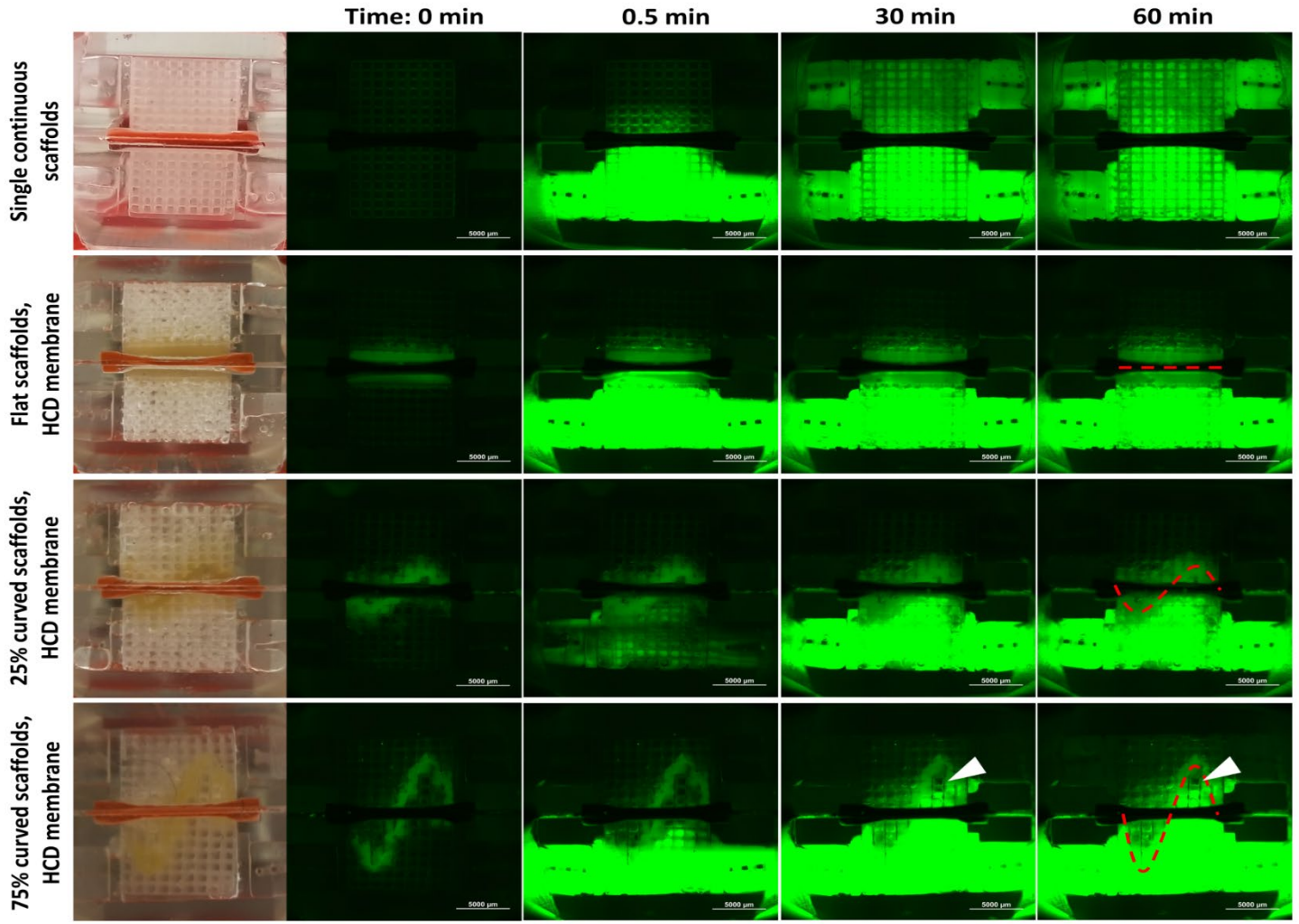
Dual Chambered Bioreactors for Stratified Cultures

3D printed scaffolds to mediate transport across the bioreactor







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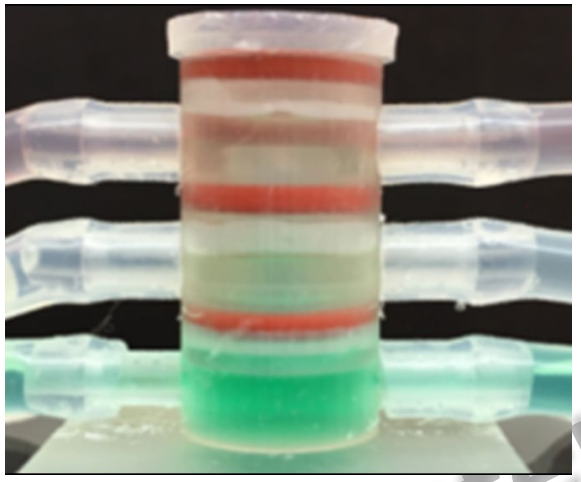
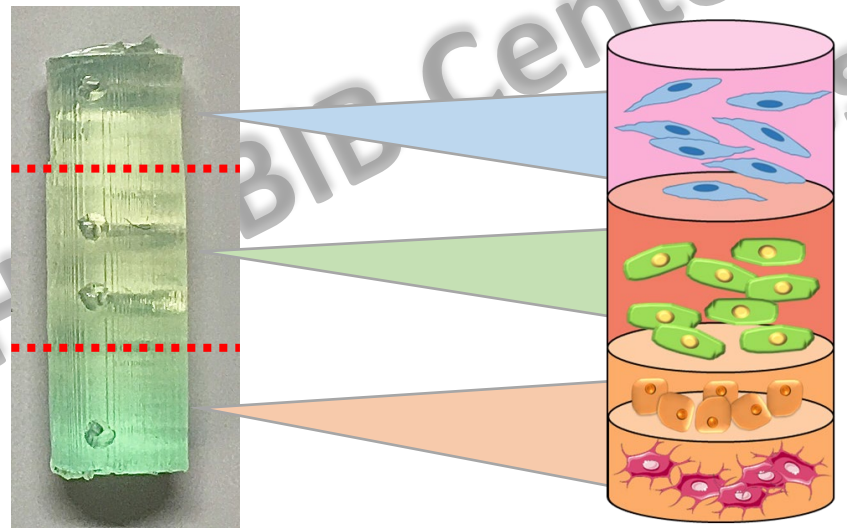
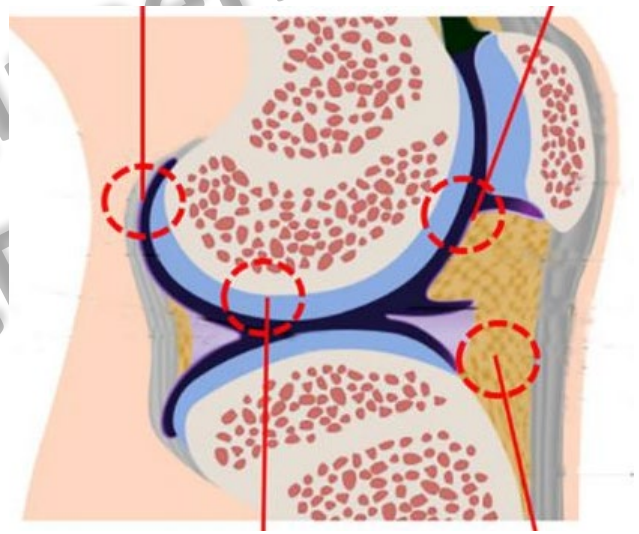
Center for Engineering Complex Tissues

Chambered Bioreactors for Tissue/Organ Culture



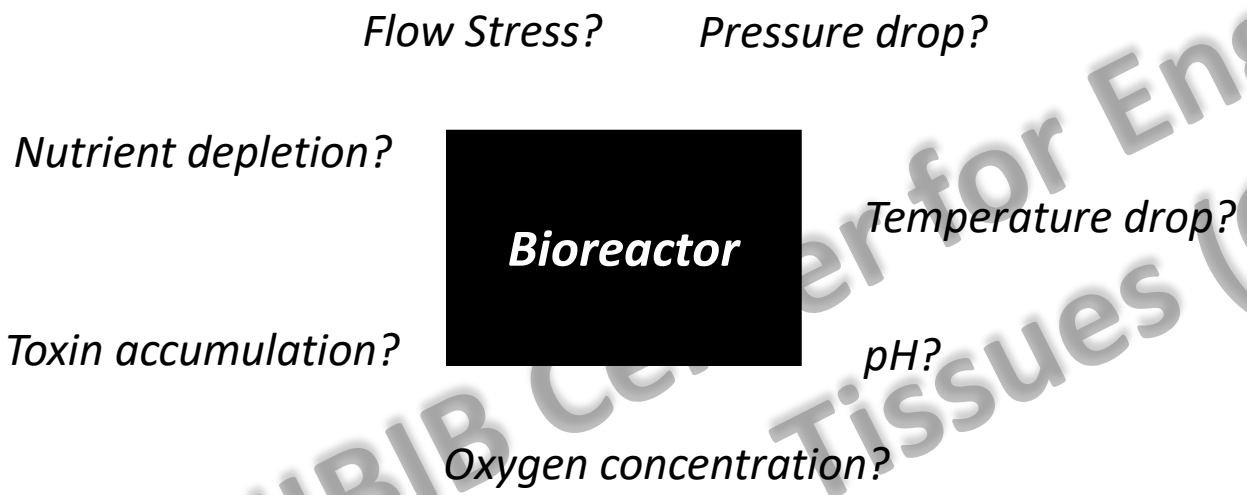


Osteochondral Tissue Engineering



- Study of gradient tissues via co-culture
- Distinct microenvironments to promote function-specific differentiation of cells

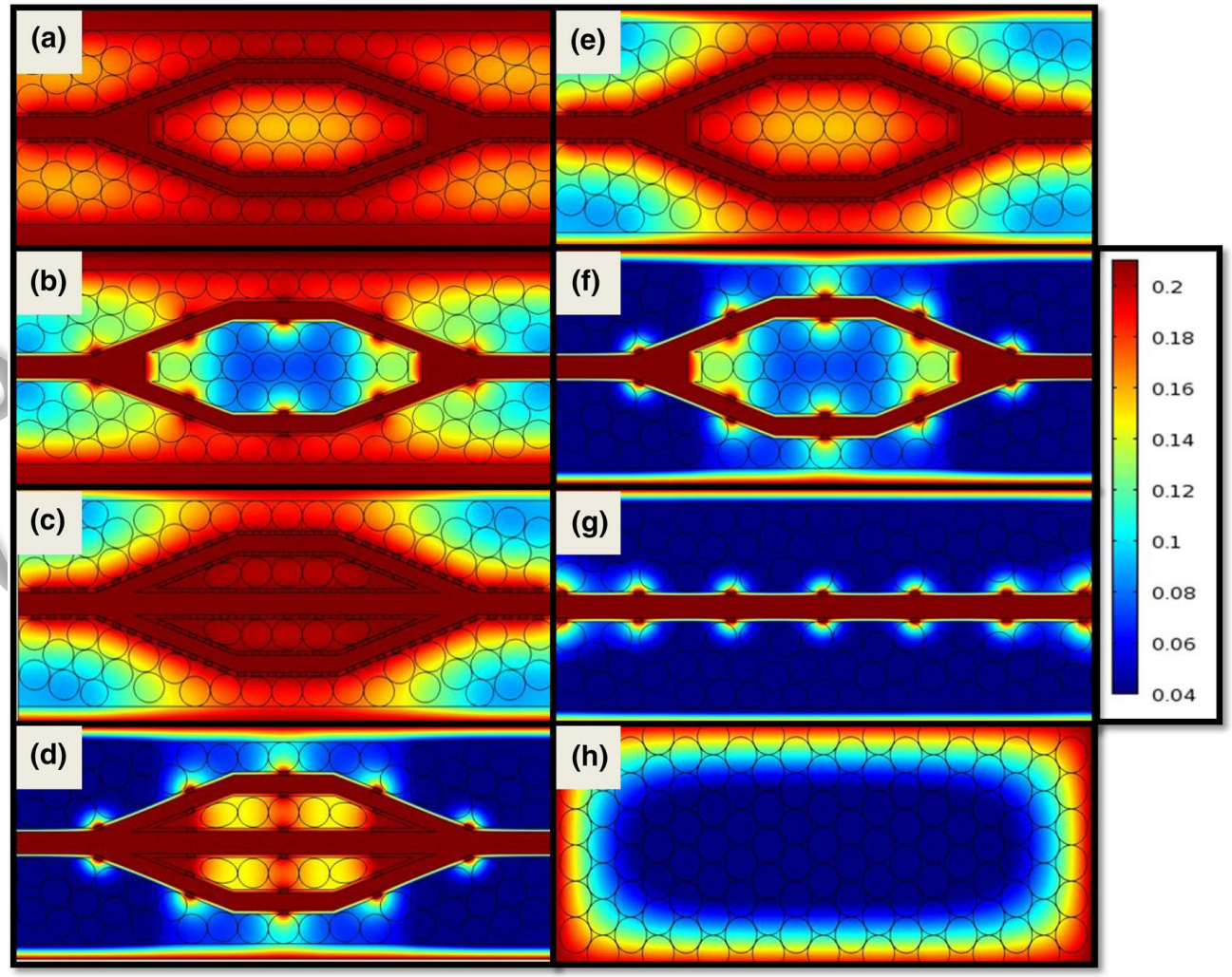
Leveraging CFD for Bioreactor Design Optimization



- Useful to estimate parameters prior to bioreactor design
- Computational fluid dynamics (CFD) methods for design optimization
  - Size
  - Geometry
  - Flow rate
  - Cell density
  - Media volume
  - …

Leveraging CFD for Bioreactor Design Optimization

- Engineering pore size and vascular geometry for O_2 diffusion through a 3D scaffold



Ball et al., Annals of Biomed Eng., Vol. 44. No. 12, Dec 2016, pp. 3435-3445

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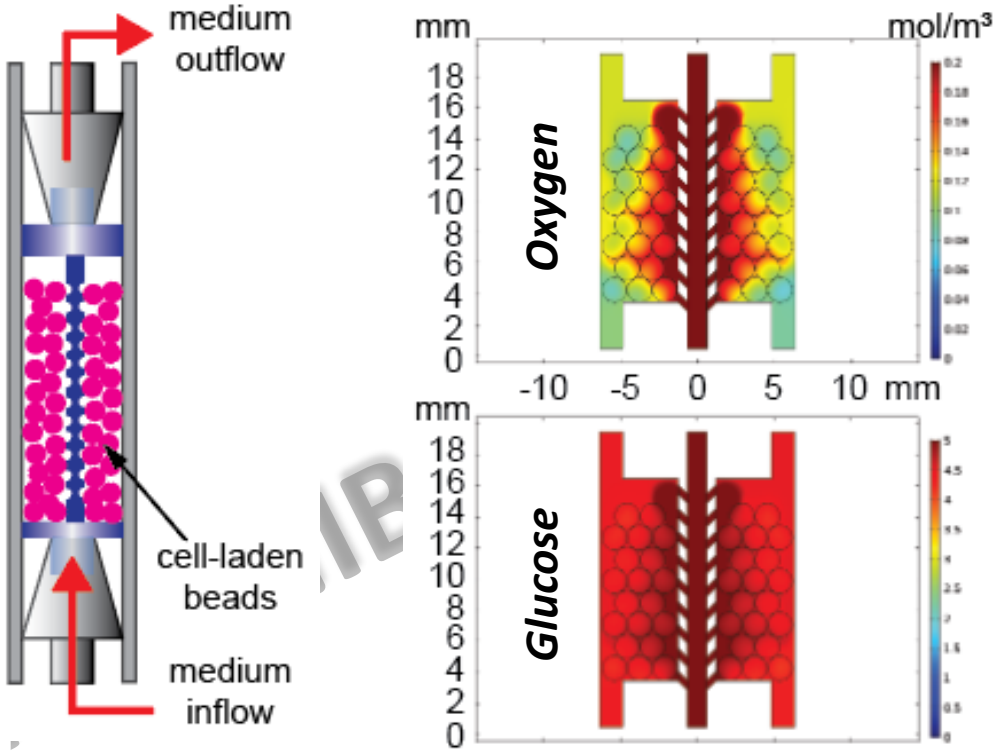
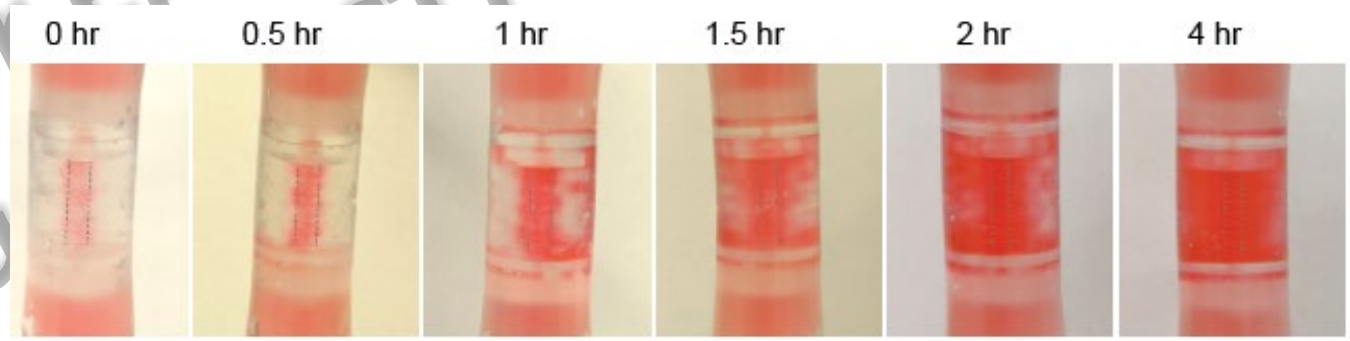
Leveraging CFD for Bioreactor Design Optimization

- Validating nutrient availability under flow

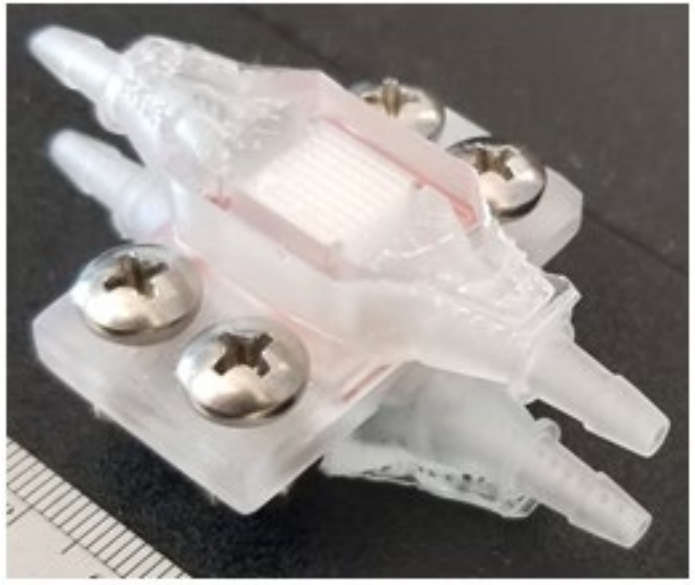


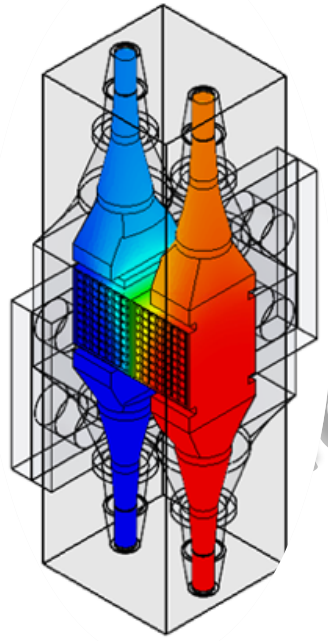


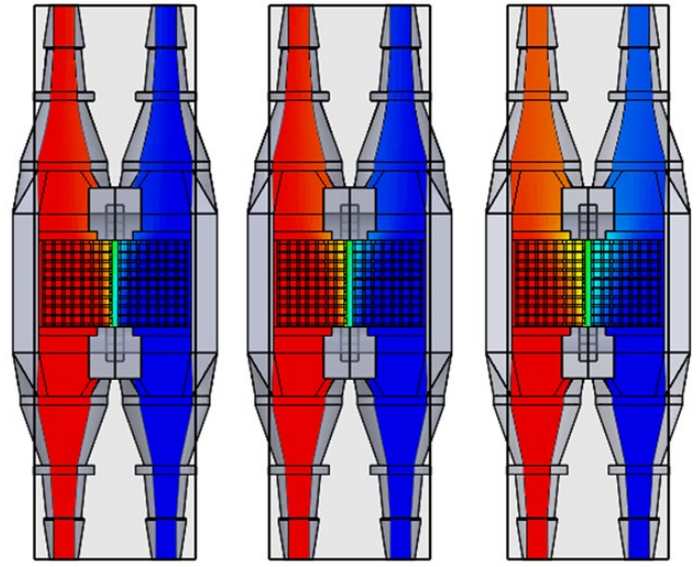
Yang et al., Tissue Eng. Part A, 2020

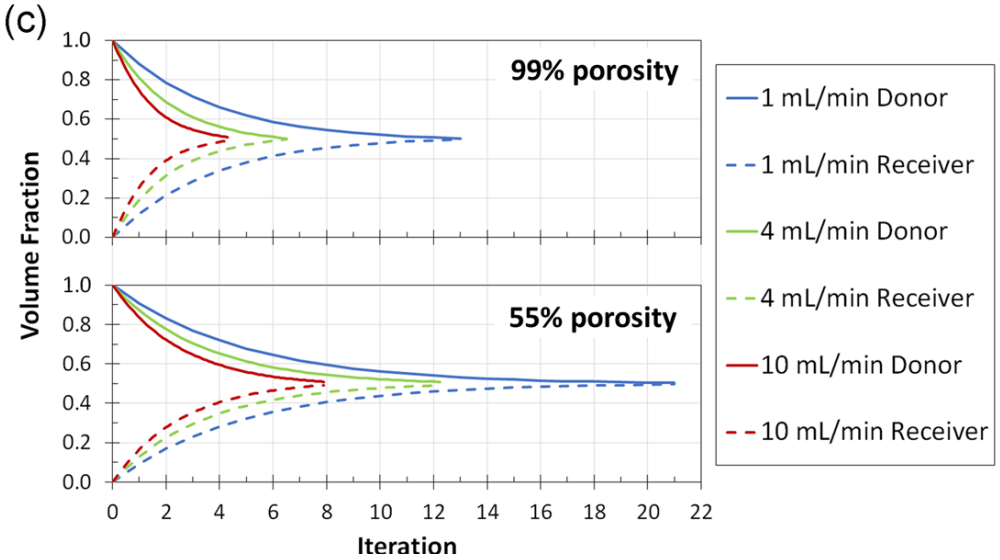
Leveraging CFD for Bioreactor Design Optimization

- Investigating transport properties within the bioreactor chamber









Navarro et al., Biotechnology and Bioengineering. 2019;116:3253–3268

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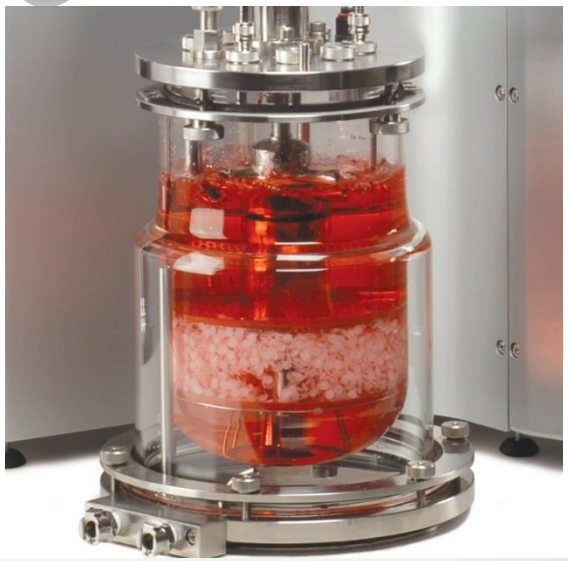
Advanced Bioreactors in Regenerative Medicine

- Large-scale, cost-effective, and reproducible production of cells or cellular products
- Beyond the ‘black box’
  - Real-time assessment and evaluation of cell and tissue maturation
- Real-time imaging
  - Non-invasive
  - µCT, fluorescence, luminescence
- In-situ control over cell biology
  - Cellular-editing for disease modeling
- Integrating automation with shifting processing conditions



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