Bioreactors

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IIRC, Kyung Hee University

Bioreactor

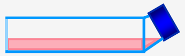
2017 Conference Program
“ Scale-up and Manufacturing of Cell-based Therapies V”
ECI (Engineering Conference International), San Diego, USA

Up-Stream & Down-Stream Processing

KHU

부착성 줄기세포 배양의 Scale-up 이슈

개발방향 1.
병렬 배양



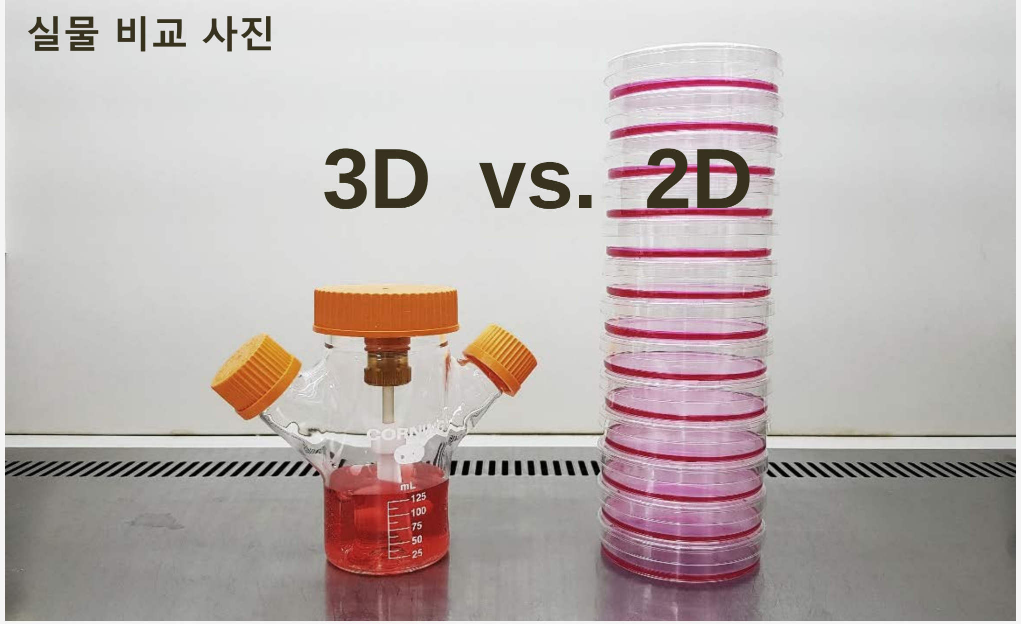
Standard 2D culture

Multiplate parallel culture

Thermo Scientific - Nunc Cell Factory

CellCultivator
(Fraunhofer, Germany)

개발 방향 2.
배양 차원 확장



Corning Spinner flask	VS	Petri dishes (100 mm)
1 bottle	# of vessel	13 dishes
125mL	Media volume	130mL
0.5~0.6 L	Vessel volume	1.76 L
Once	# Handling repeat	13 times

예시) 동일 표면적 기준 MC-based 3D vs. 2D 비교

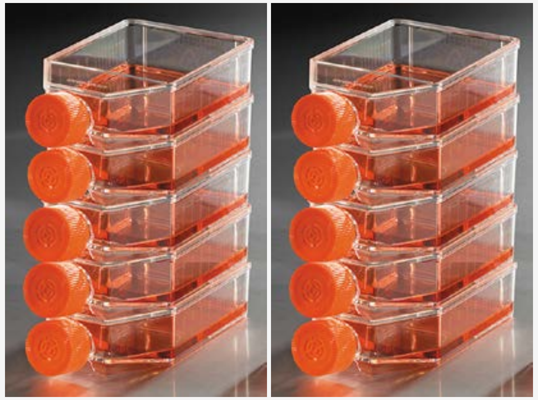
- MC 2.5 g in 125 mL spinner flask ≈ 100 mm dish 13개
- 상용 Corning disposable spinner flask 3 L ≈ 100 mm dish 312개

Scale-up Production of Stem Cells by 3D Cell Culture Platform

Standard 2D culture

5X10^7 cells

175T flask (10x) + 250ml media



Solohill-based 3D culture

5X10^7 cells

4.86g microcarriers/250ml media

2.2356X10^6 microcarriers/4.86g

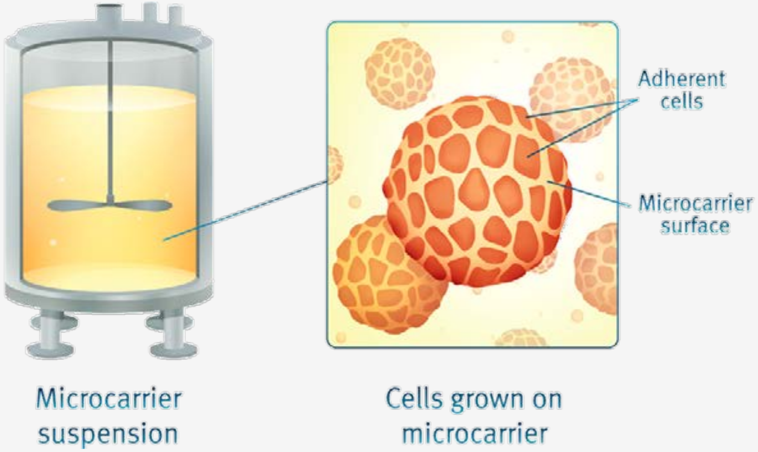
Volume comparison

(equivalent surface area)

175T Flask: 4.5 L

10 * 450ml

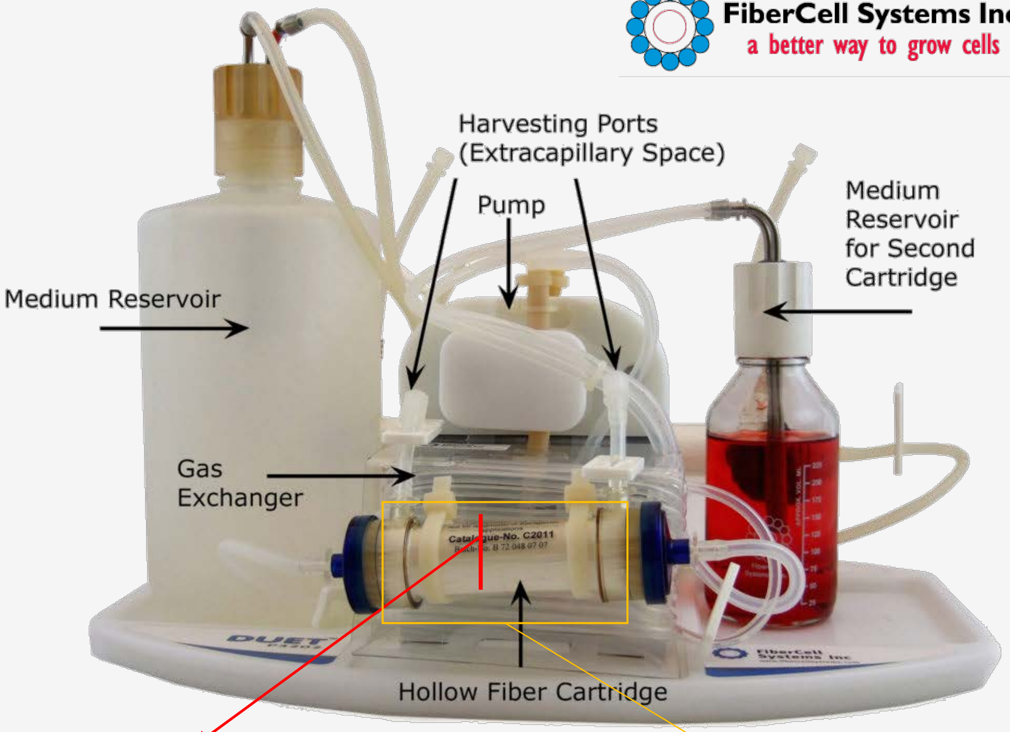
Spinner Flask: 450 ml

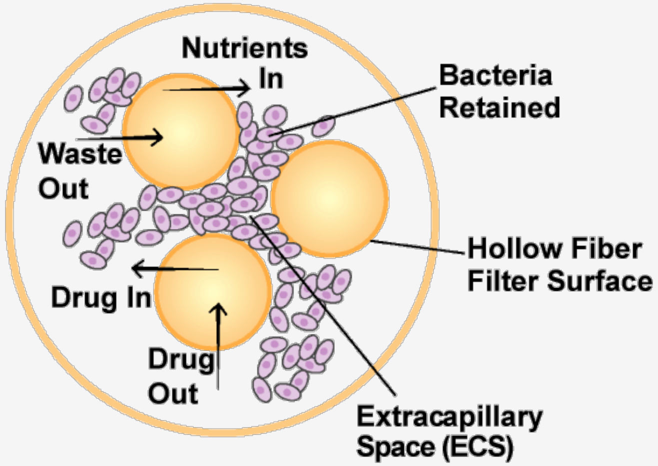


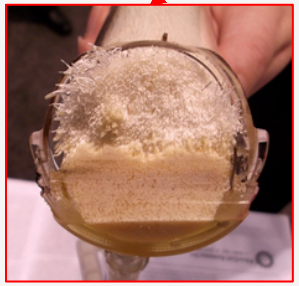
Scalable 3D culture platform

Fixed Bed Bioreactor - Hollow fiber

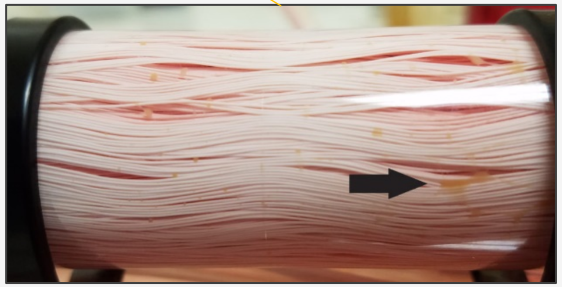
FiberCell Systems Inc.
a better way to grow cells







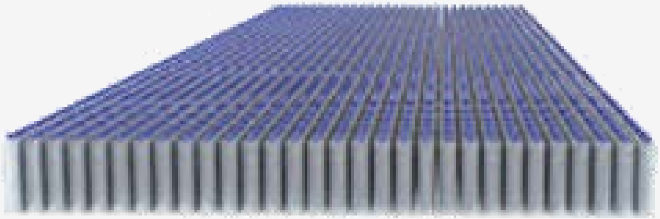
Hollow fiber의 단면



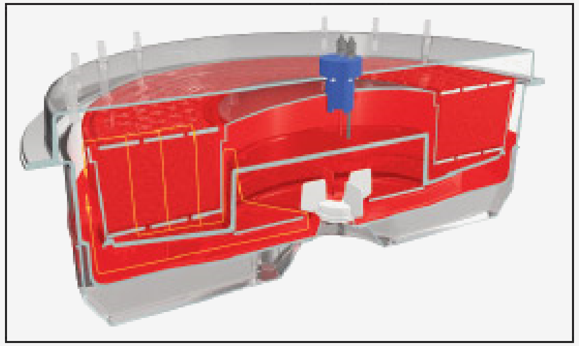
Hollow fiber 표면 세포가 엉겨있는 모습

<Fiber Cell 단면>

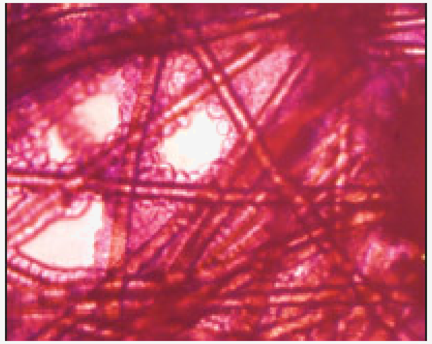
iCELLis 500 - Fixed Bed Bioreactor



iCELLis 500 모델 바이오리액터 탱크 1개(500m^2)는3,000개의 Roller bottle(각각 1700 cm^2)과 동일한 표면적을 가지고 있음



iCELLis 500 바이오리액터 내에서 배지의 흐름 (60-70L)



iCELLis 시스템 캐리어에 부착되어 자라는 MDCK 세포의 현미경 사진

iCELLis 500 바이오리액터의 전체 모습

마이크로캐리어 기반 3차원 확장배양

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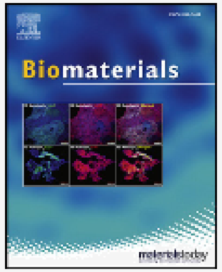


Review

Large-scale production of stem cells utilizing microcarriers: A biomaterials engineering perspective from academic research to commercialized products

Hossein Tavassoli [a, b], Sanaz Naghavi Alhosseini [c, d], Andy Tay [e], Peggy P.Y. Chan [b], Steve Kah Weng Oh [f], Majid Ebrahimi Warkiani [g, h, *]

[a] Adult Cancer Program, Lowy Cancer Research Center, Prince of Wales Clinical School, University of New South Wales, Sydney, NSW, 2052, Australia
[b] Department of Biomedical Engineering, Swinburne University of Technology, Hawthorn, Victoria, 3122, Australia
[c] Biomaterials Group, Department of Biomedical Engineering (Center of Excellence), Amirkabir University of Technology, Tehran, Iran
[d] Department of Nanotechnology and Tissue Engineering, Stem Cell Technology Research Center, Tehran, Iran
[e] School of Biomedical Engineering, UCLA, Los Angeles, USA
[f] Stem Cell Group, Bioprocessing Technology Institute, A*STAR (Agency for Science, Technology and Research), 20 Biopolis Way, #06-01, 138668, Singapore
[g] School of Biomedical Engineering, University of Technology Sydney, Sydney, Australia
[h] Institute of Molecular Medicine, Sechenov First Moscow State University, Moscow, 119991, Russia

마이크로캐리어 기반 3차원 확장배양

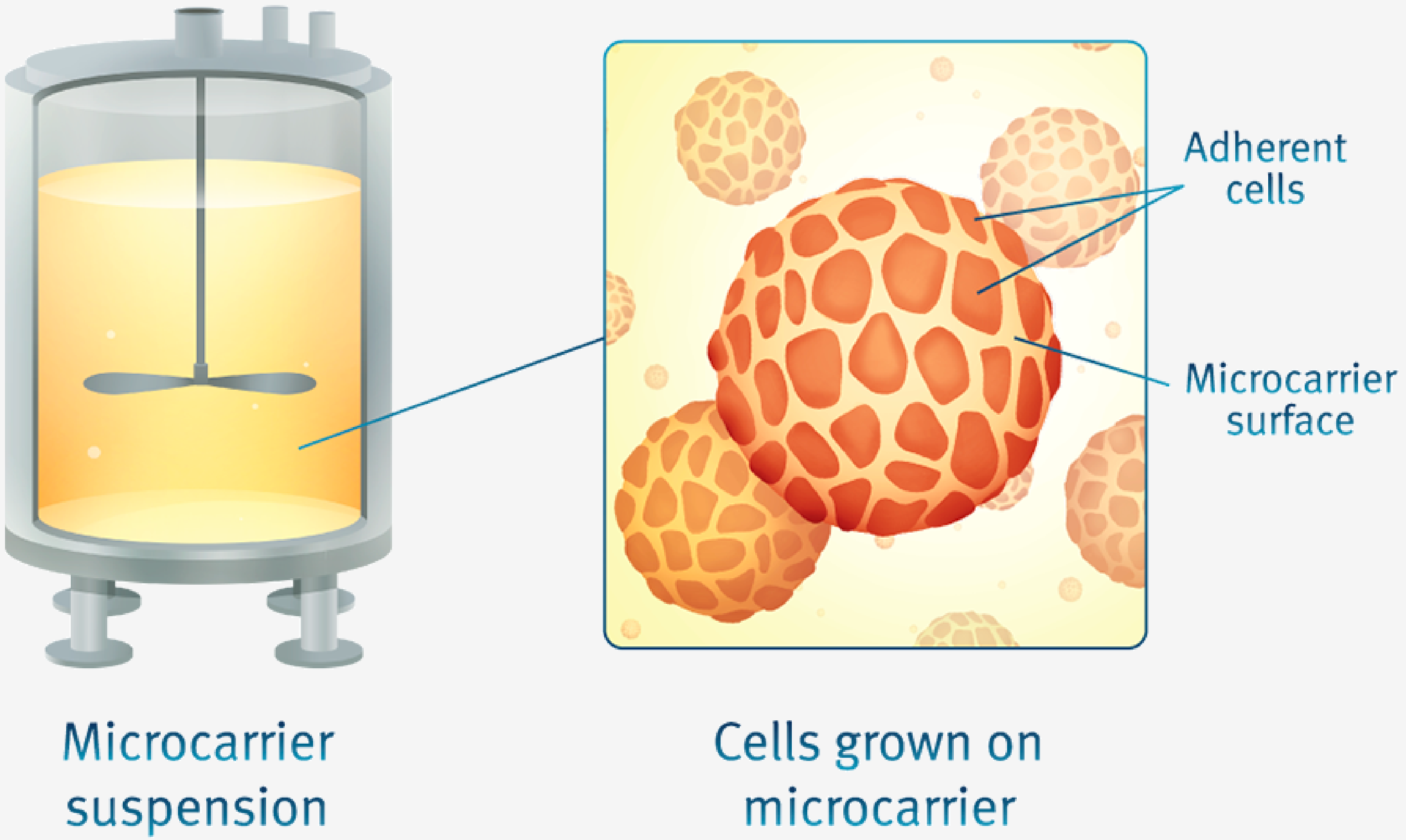
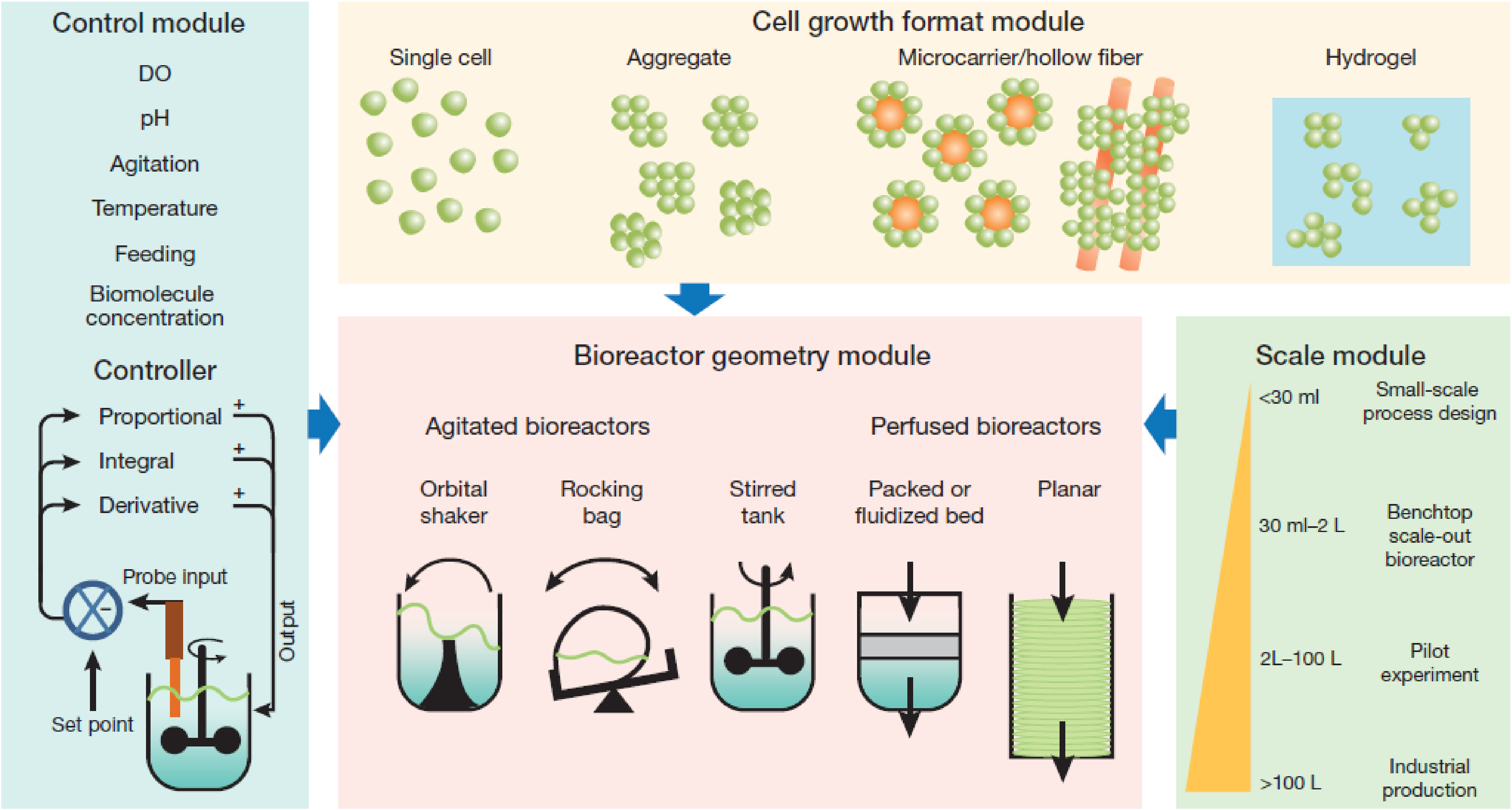
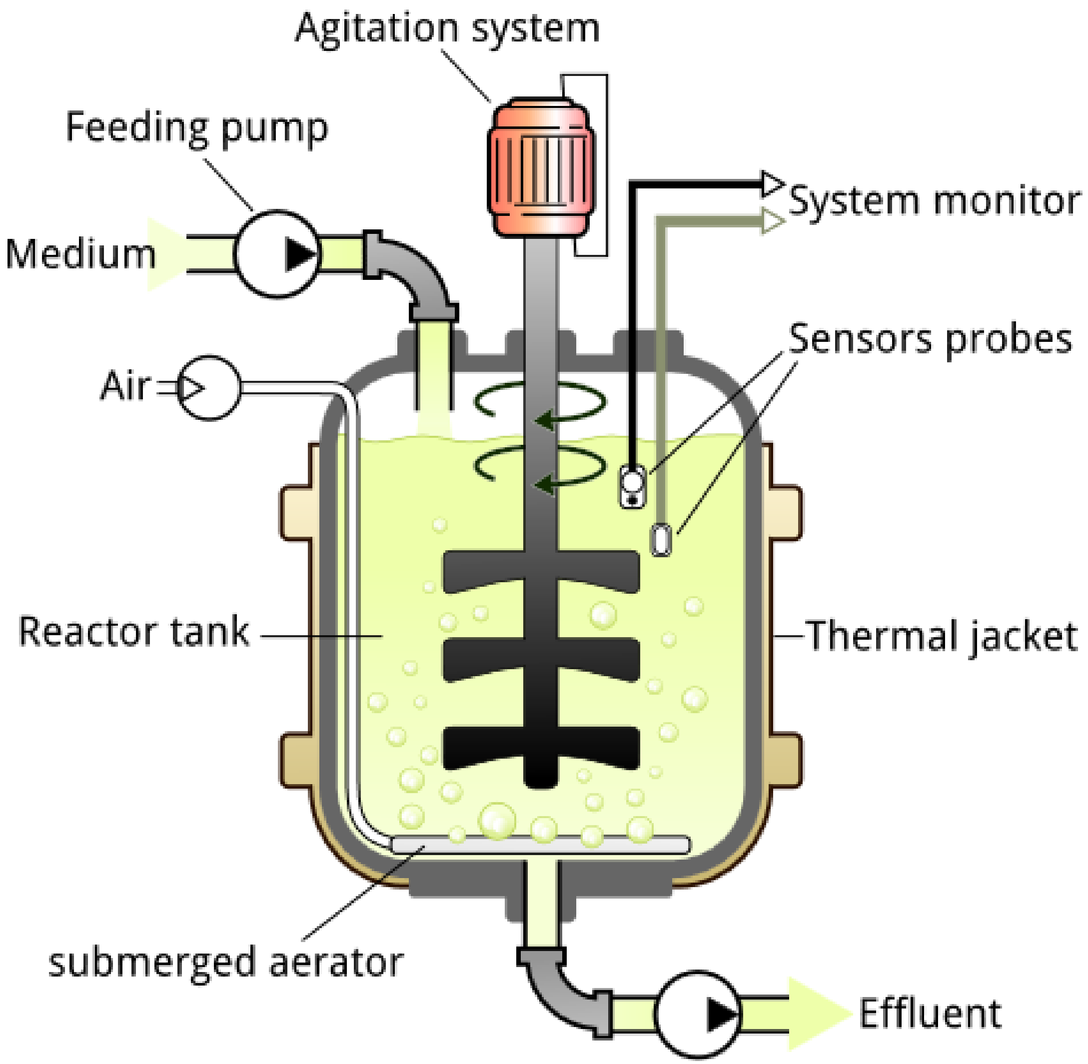


Image source: https://**chemometec.com**/counting-cells-microcarriers

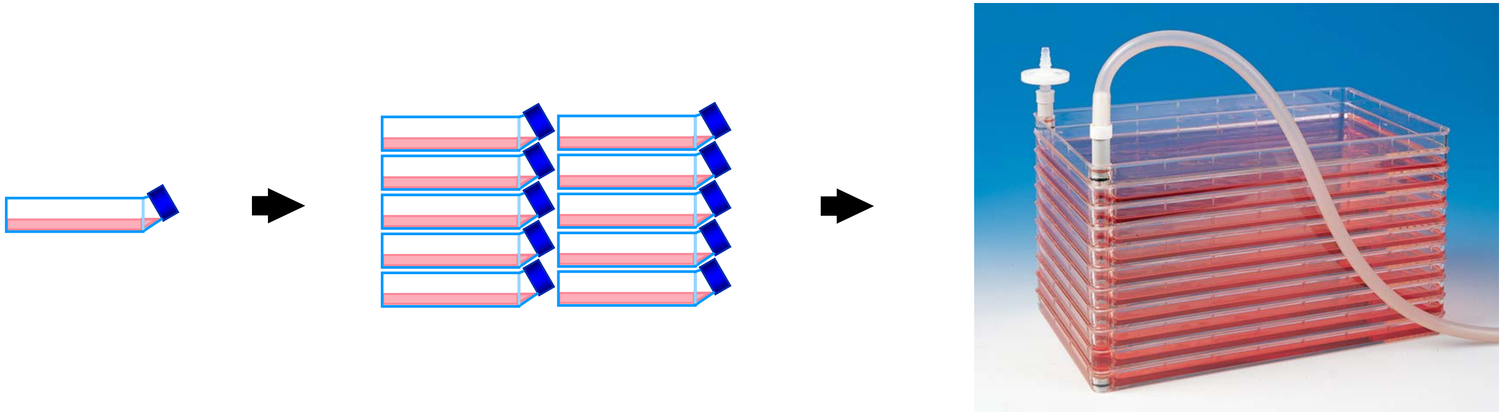


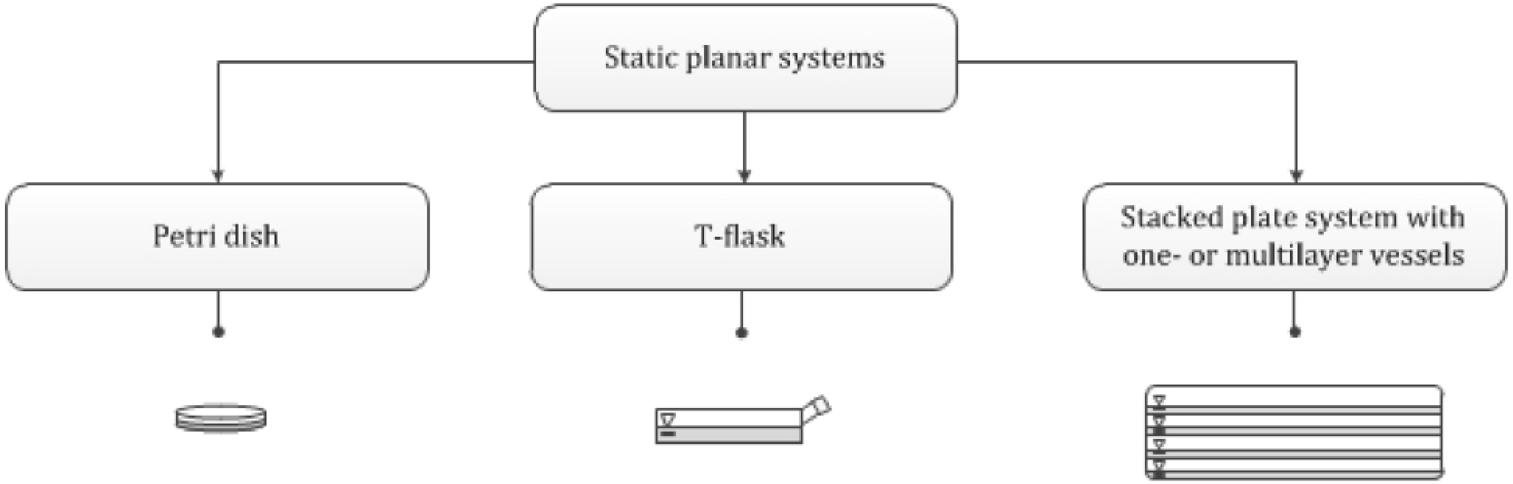
[Lipsitz, timmins, & Zandstra, "Quality cell therapy manufacturing by design" Nature BT 34, 393-400, 2016]
http://www.nature.com/nbt/journal/v34/n4/pdf/nbt.3525.pdf

Mass Culture



Scaling up of Stem Cell Culture



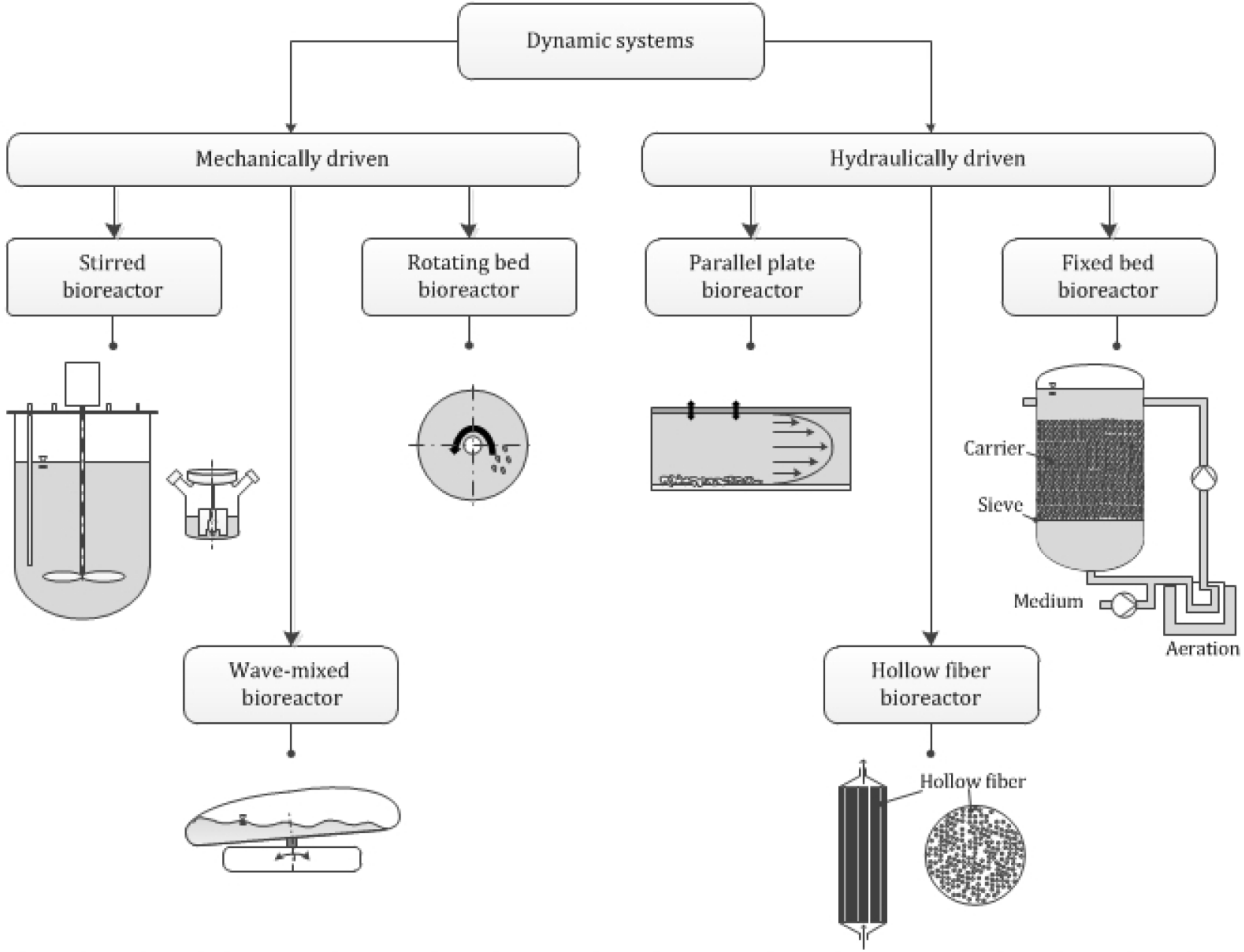


Dynamic Mass Culture

“How to use computational fluid dynamics in the development of cell therapeutics”

Computational fluid dynamics (CFD)

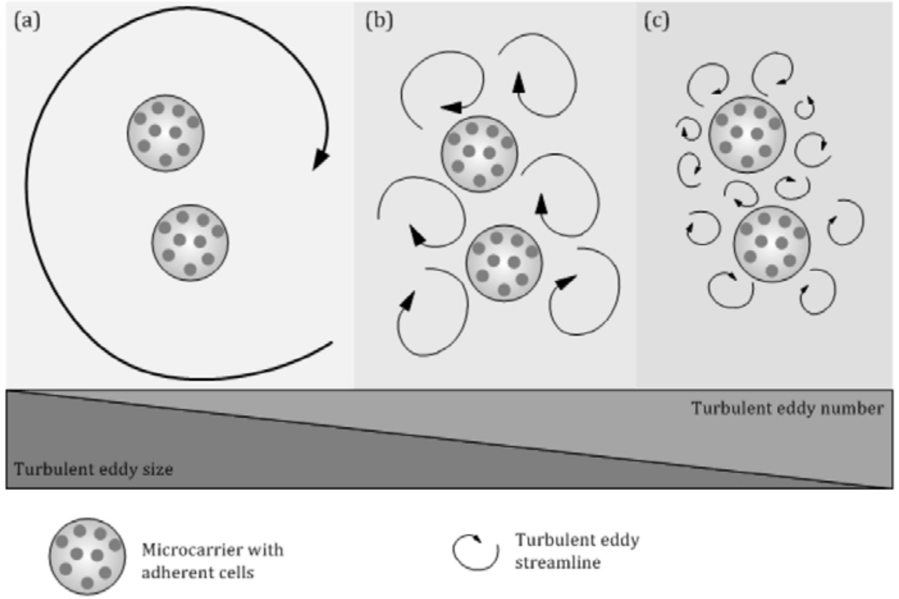
[Valentin Jossen 2014 Cells and Biomaterials in RM Chapter5]



Stirred Bioreactor

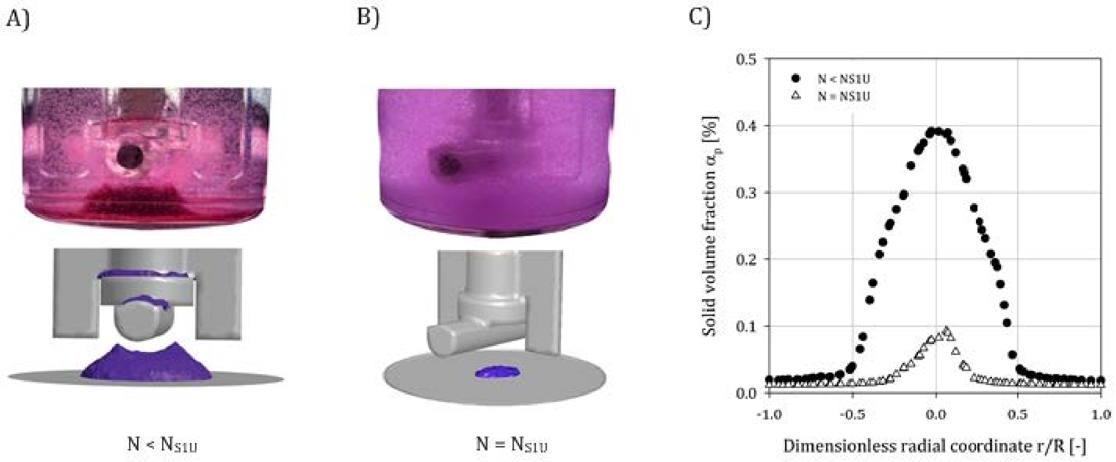
*** Shear forces on microcarriers in a turbulent flow**

- Microcarrier – Eddy interactions



* Experimentally obtained biological information are coupling with simulation data – shows vision

- Computational simulation predicts the real situations quite well.
- Have worked with various manor cell expansion system developers.



S1U: represent the lowest impeller speeds required for the MCs to become just-suspended

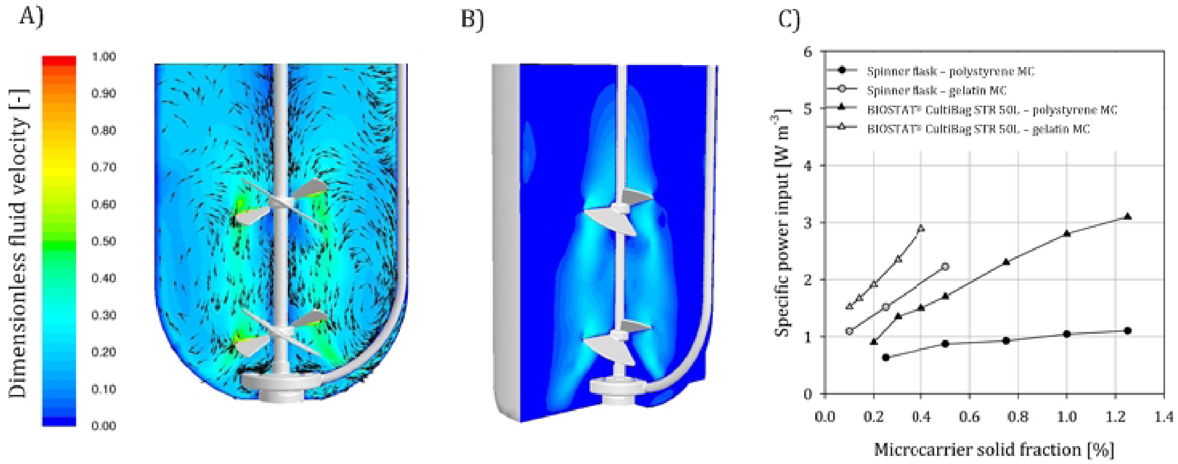
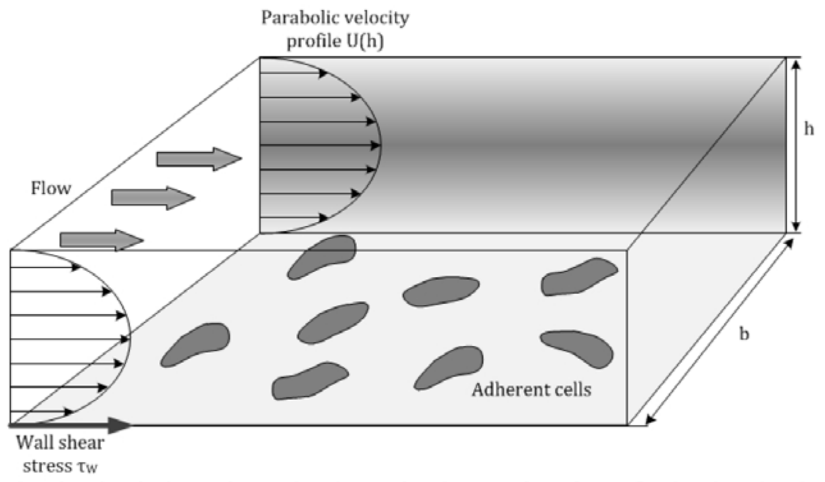


Figure 10. Fluid flow conditions and required specific power inputs for the N_{S1U} criterion in the BIOSTAT® CultiBag STR 50L. The contour and vector-plots are given along the mid-reactor plane. The contour plot (A) illustrates that the induced fluid flow in the BIOSTAT® CultiBag STR 50L is primary axial with two flow loops, whereby the axial fluid velocities (B) impinge on the reactor bottom and enable the microcarriers to swirl up. (C) The specific power inputs required to fulfill the N_{S1U} criterion are in a comparable range for both the spinner flask and the BIOSTAT® CultiBag STR 50L.

Parallel Plate Bioreactor

* Wall shear stress acting on adherent cells in a flow chamber with parallel plates

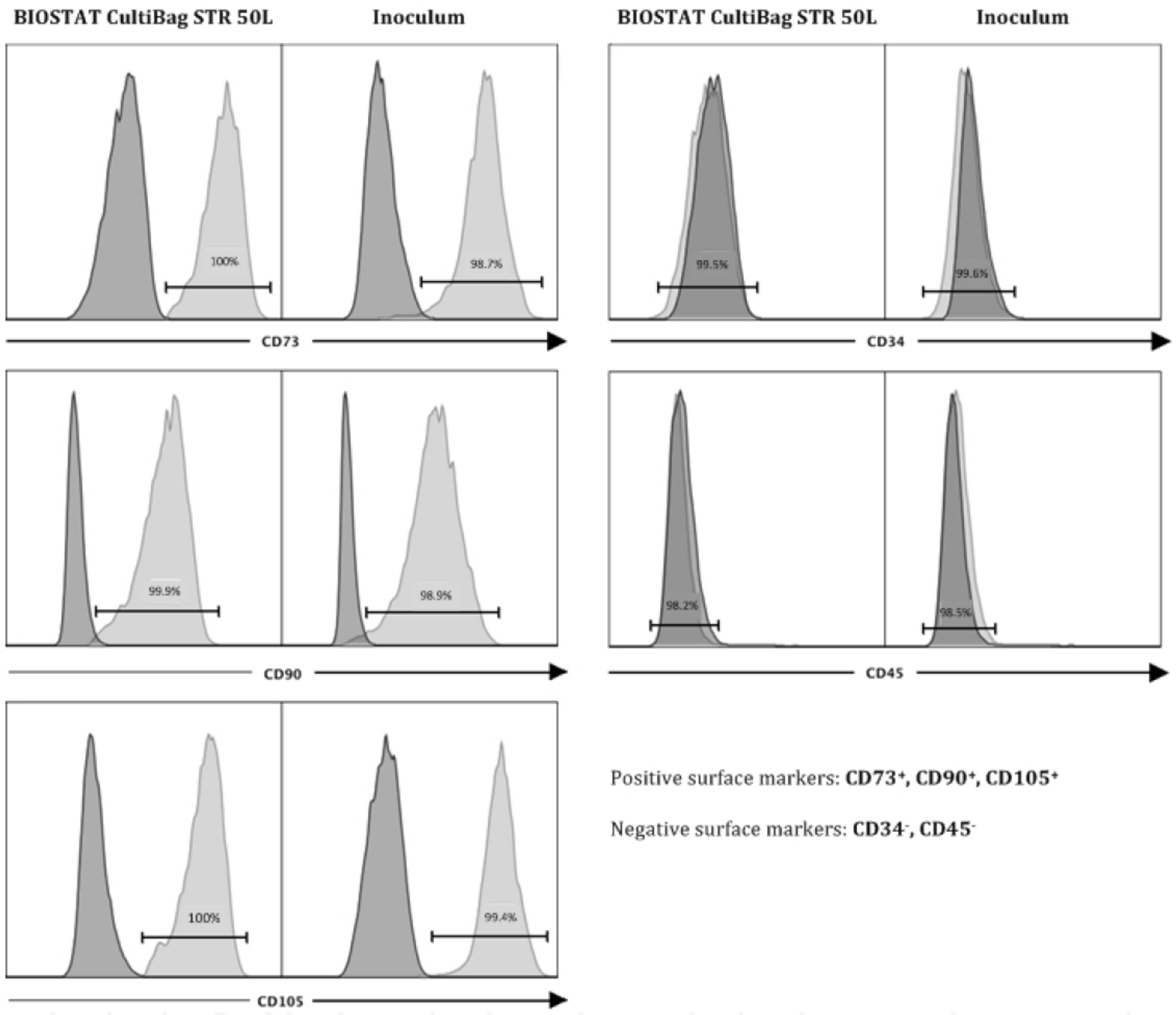


"How to use computational fluid dynamics in the development of cell therapeutics"

Computational fluid dynamics (CFD)

[Valentin Jossen 2014 Cells and Biomaterials in RM Chapter5]

Quality Control after Mass Culture



Positive surface markers: **CD73⁺, CD90⁺, CD105⁺**

Negative surface markers: **CD34⁻, CD45⁻**

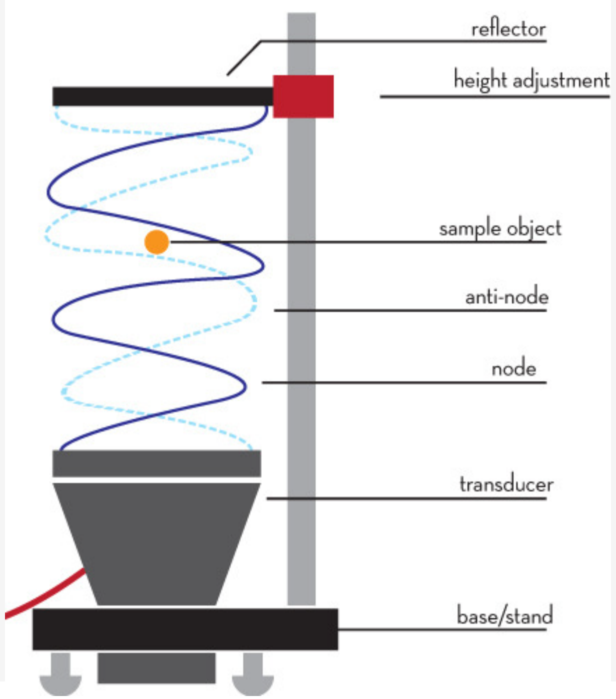
환자 맞춤형 CAR-T: 다품종 소량생산 요구

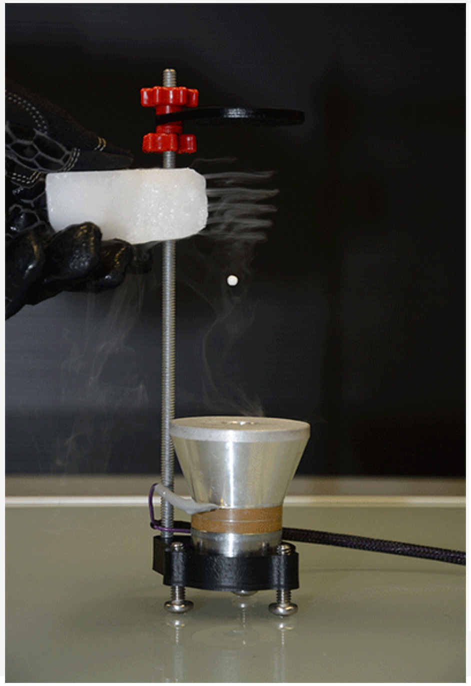
- **Cocoon Bioreactor (OCTANE Biotech Inc)**

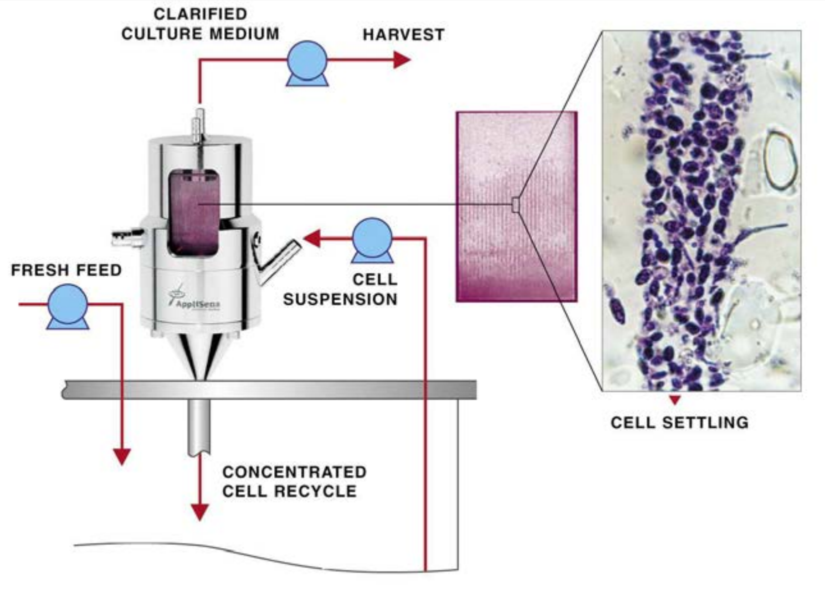
Devices for Down-Stream Processing

BioSep <Applikon Biotechnology> BioSep: www.applikon-bio.com/en

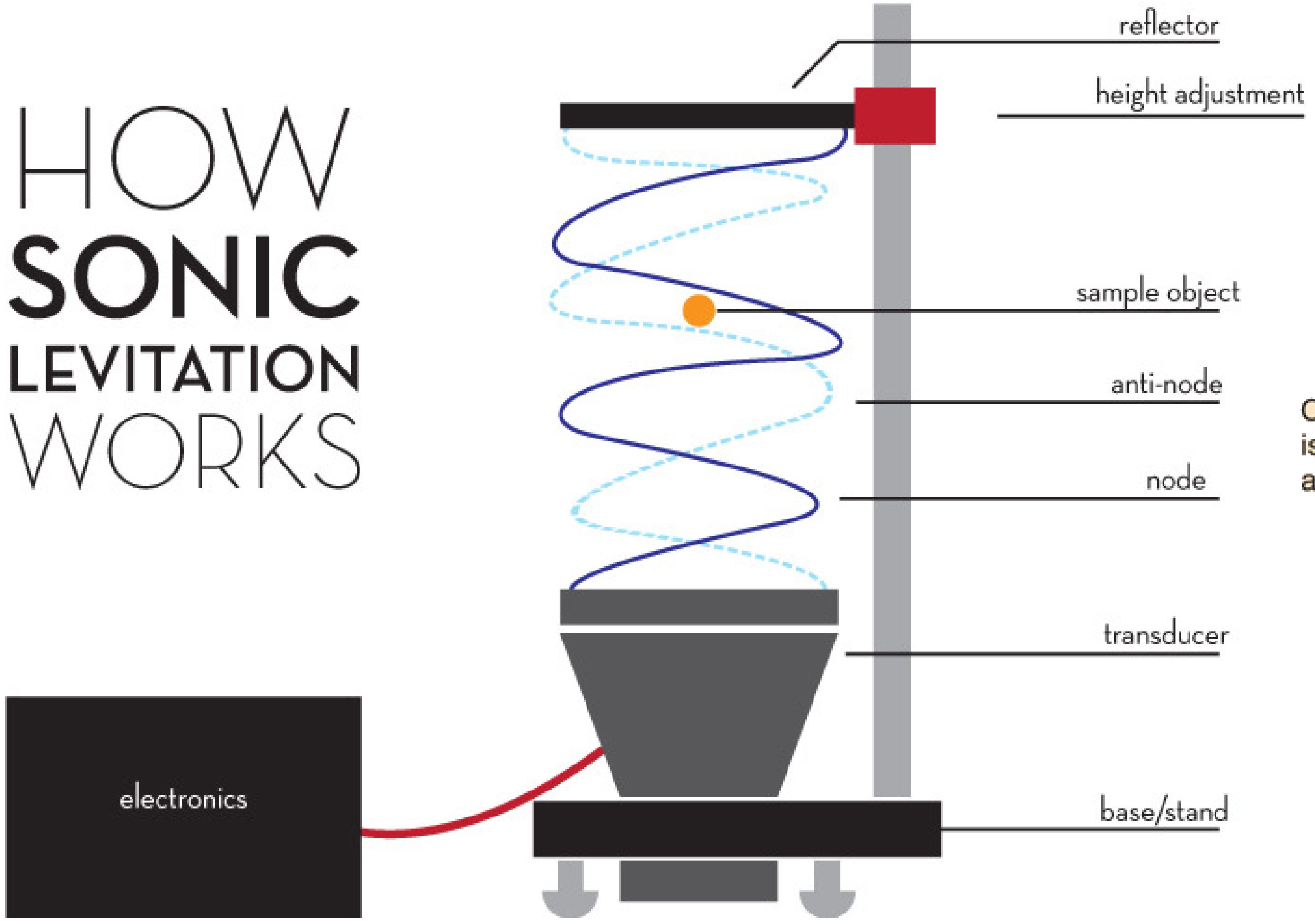
Retention system for perfusion, concentration and washing



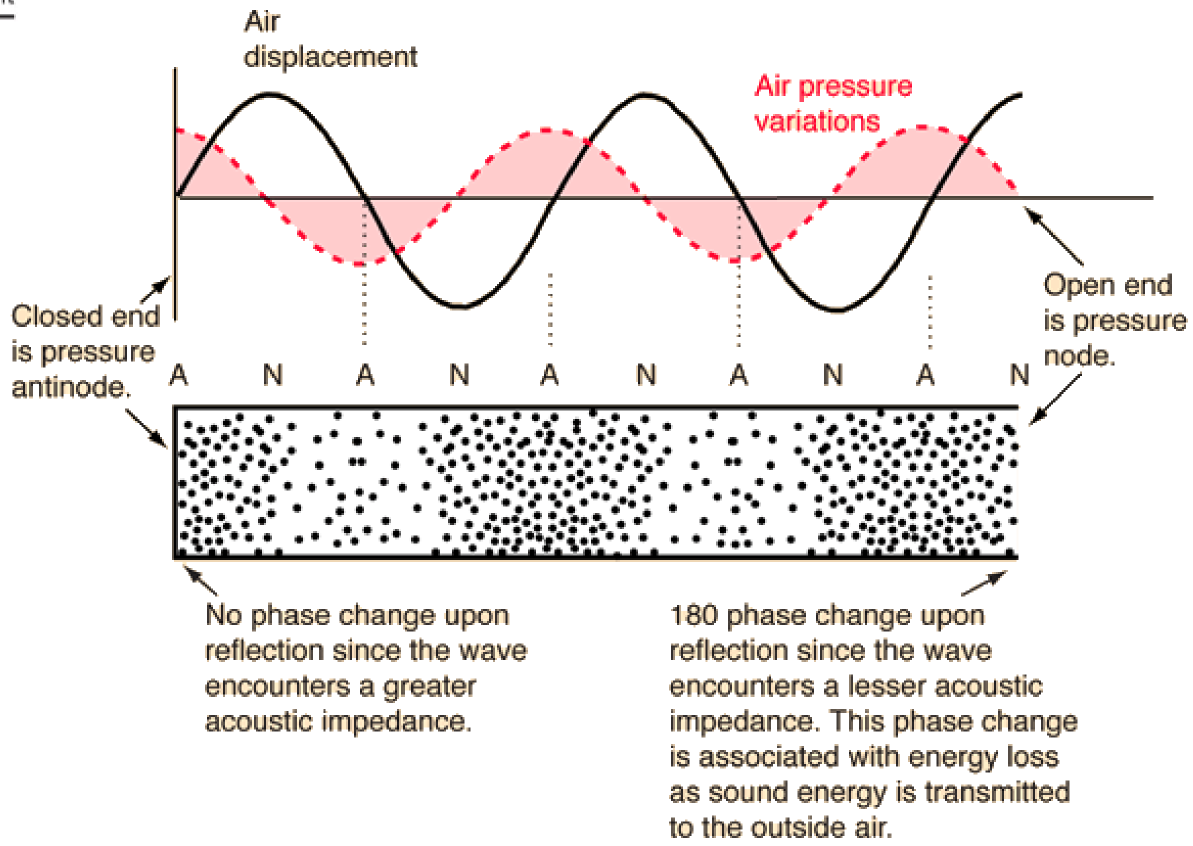




Typical configuration of acoustic cell retention system.

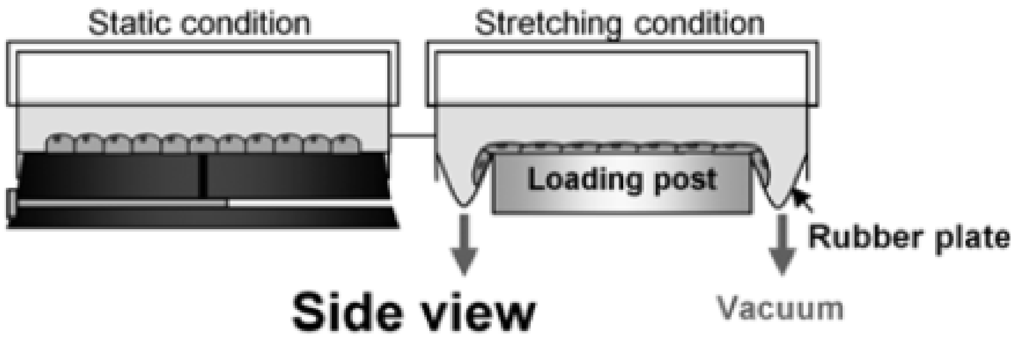


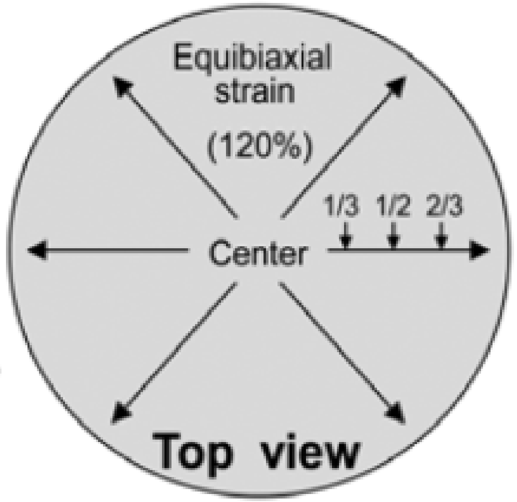
Production of a standing wave in an air column involves reflections from both the closed end and the open end of the column.



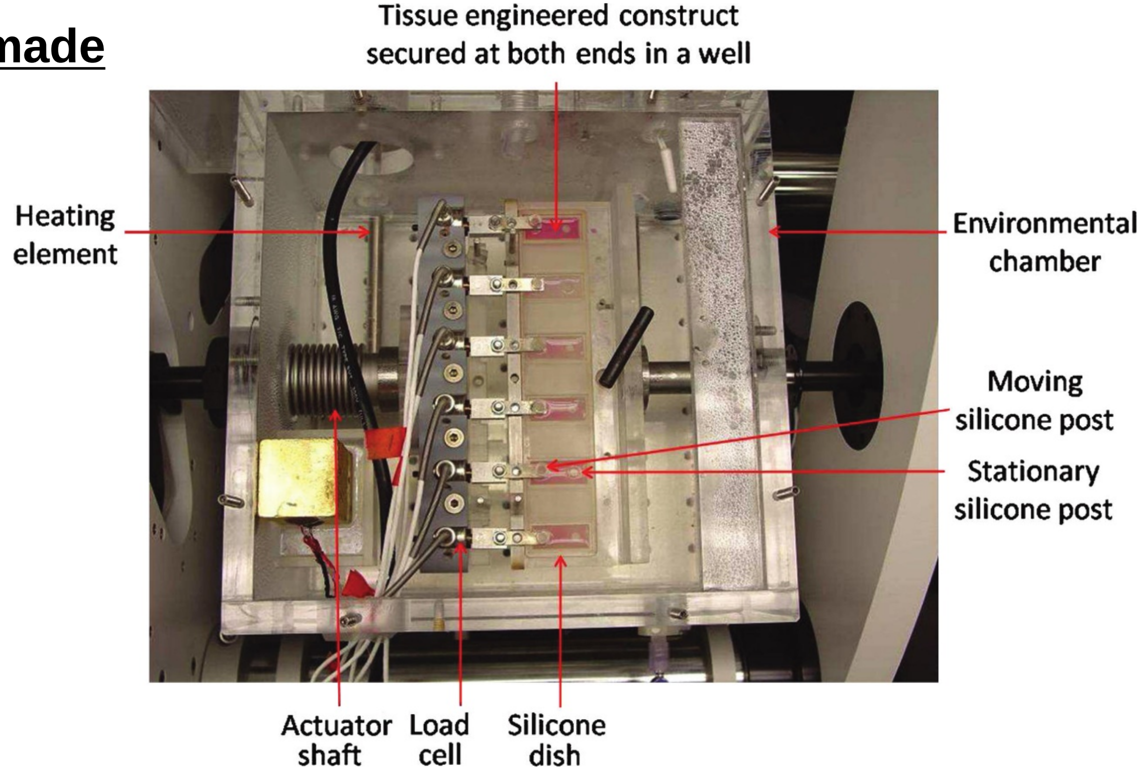
Bioreactor for tensile stimulus

FlexCell FX4000

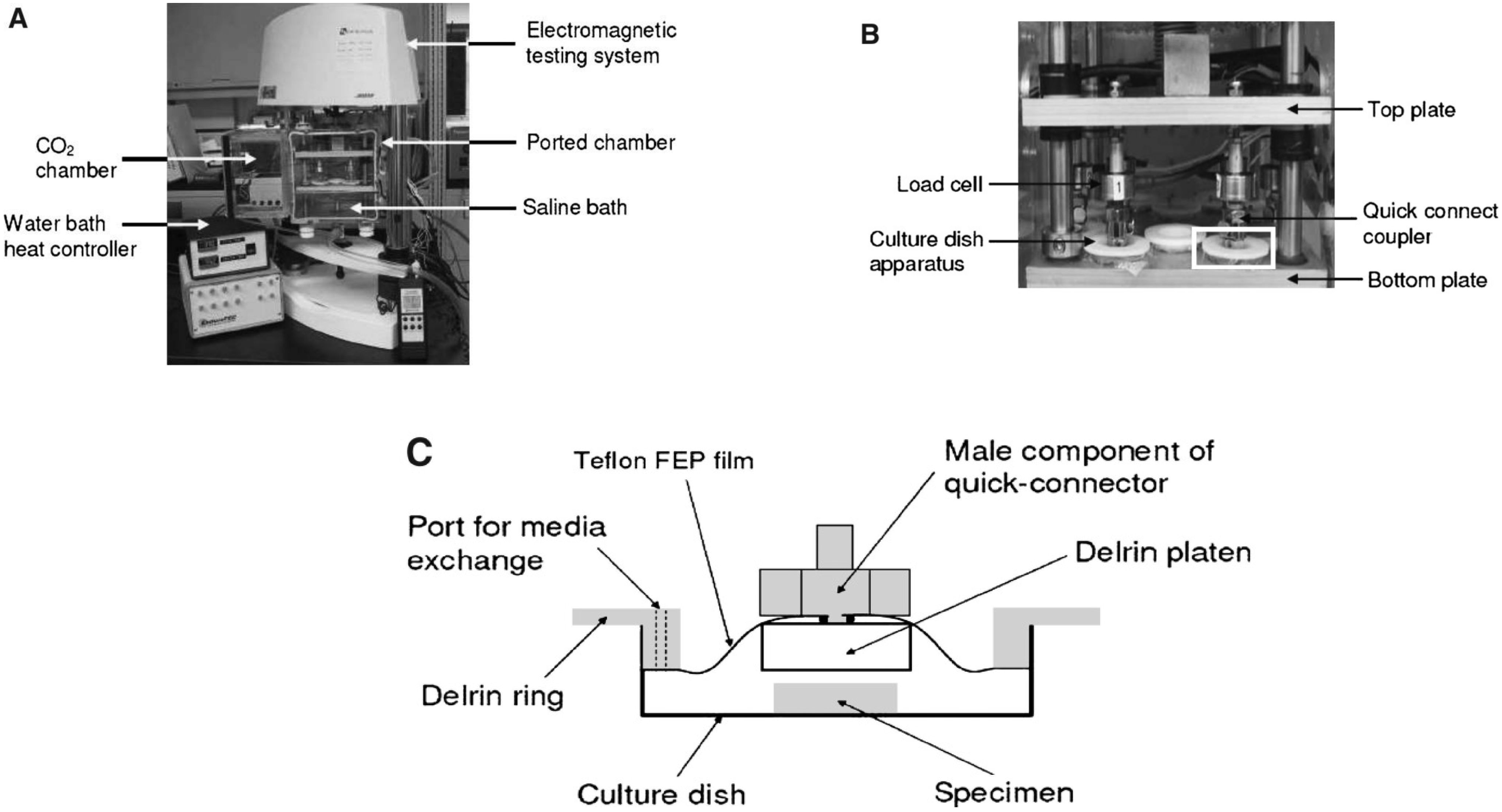




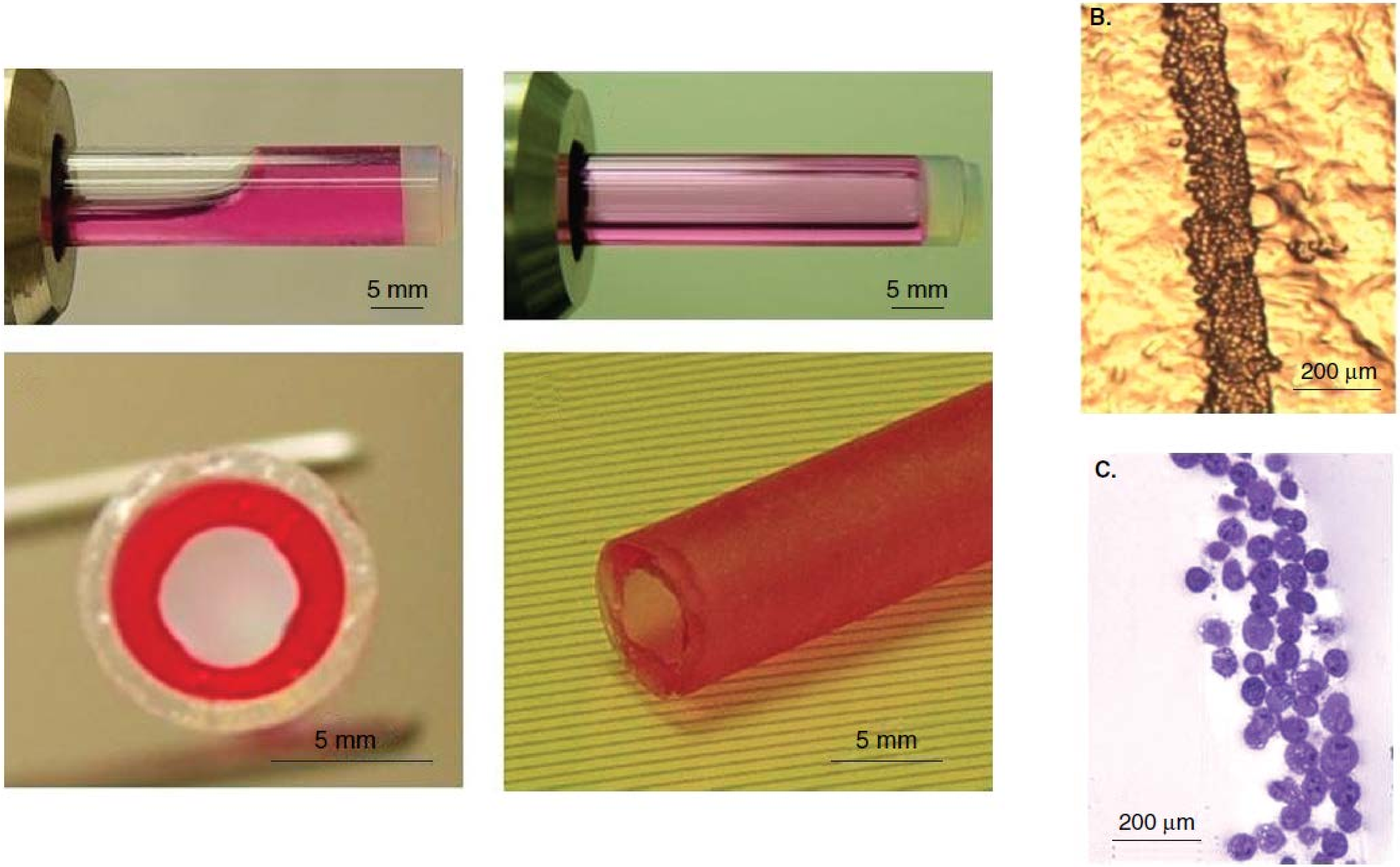
Custom-made

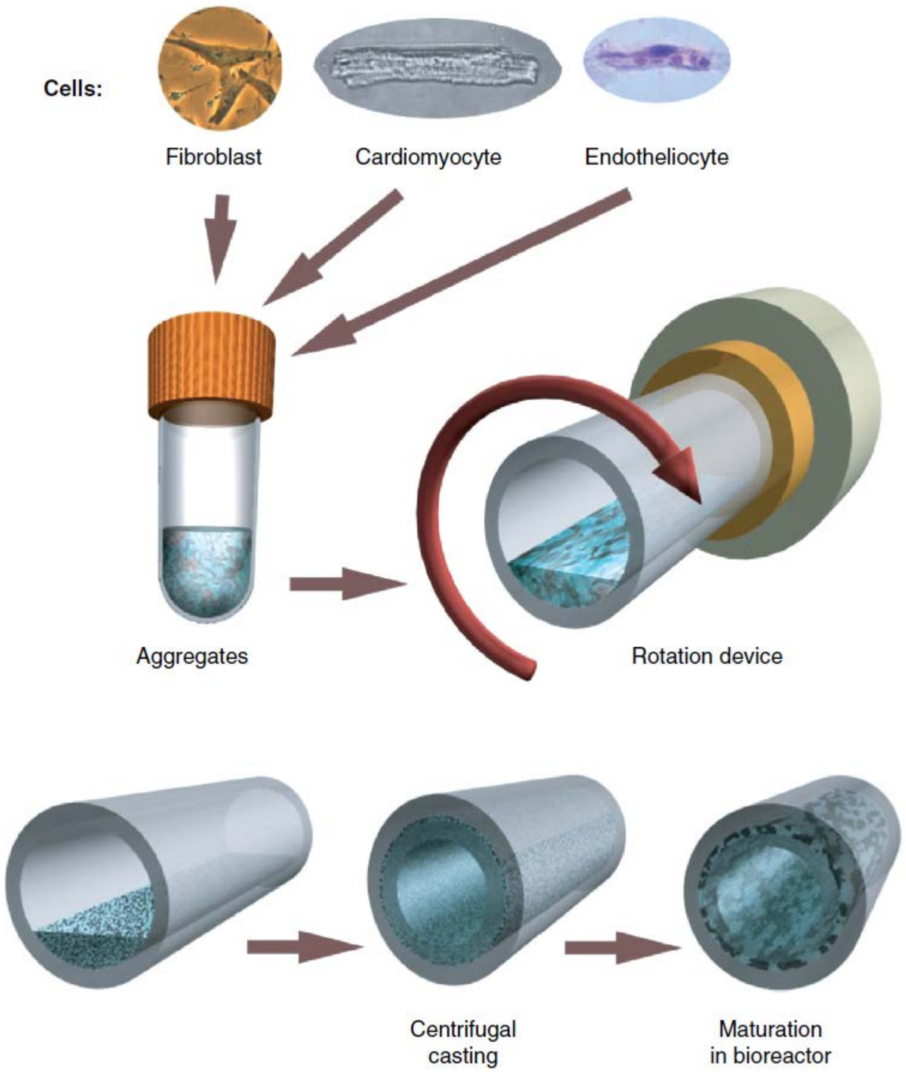


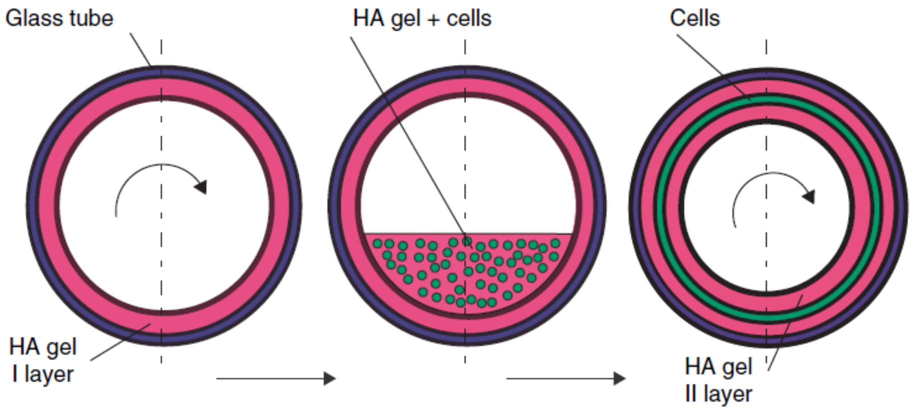
Compressive Stimulus Bioreactor



Bioreactor-Free TE




Cells:
Fibroblast
Cardiomyocyte
Endotheliocyte
Aggregates
Rotation device
Centrifugal casting
Maturation in bioreactor


Glass tube
HA gel + cells
Cells
HA gel I layer
HA gel II layer