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Successfully employing single-use bioreactors for different expression systems

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Department of Biochemical Engineering and Cell Cultivation Technique

Successfully employing single-use bioreactors for different expression systems

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http://www.lsfm.zhaw.ch/de/science/ibt-bvt.html

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Single-Use Technology: Overview

In case of single-use (disposable) bioreactors the cultivation container is only used once.

Cultivation container = Multiwell plate, tube, Erlenmeyer, flask, vessel, bag (mL-2500L)

- Non-reusable cultivation container
- FDA-approved plastics: molded systems or flexible bags
- Preassembled, gamma- or beta-sterilized

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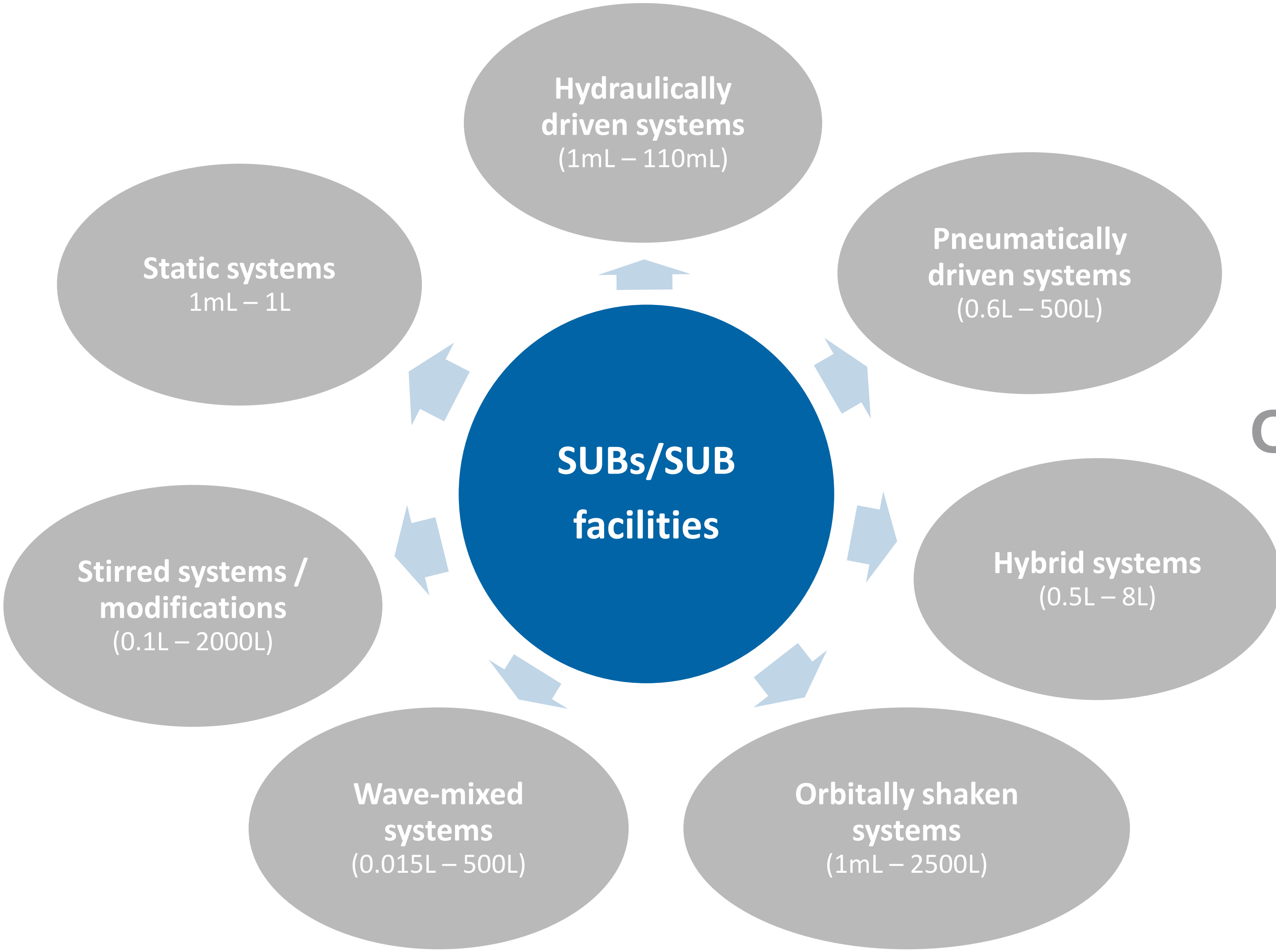
Single-use bioreactors

- Only cultivation container

Single-use bioreactor facilities

- Sensors, measurement and control unit

Single-Use Technology: Categorization



OR

Instrumentation

- Noninstrumented systems
- Less instrumented systems
- Highly instrumented systems

Single-Use Technology: Pros vs. Cons

Pros

Safer

- Decreased risk of contamination

Greener

- Reduced requirements for cleaning and sterilization

Faster and more flexible

- Easy process and product change

Cheaper

- Saving of time and costs

Smaller

- Reduced facility footprint

Cons

Weakness

- Limited scalability
- Limited standardization
- Transport sensitivity (bag)
- Increased storage requirements
- Increased waste generation
- Repetitive consumable costs
- Lead times of supply

Threats

- Supplier dependency
- Extractables and Leachables
- Instrumentation level

Single-Use Technology: Field of applications

SUBs are well-established, although they are not always optimal!

Protein-based biopharmaceuticals from mammalian and insect cells

- Screening
  (Orbitally shaken and stirred systems at mL-scale)
- Inoculum production
  (Predominance of wave-mixed systems)
- Preclinical and clinical sample production of GMP products in low- and medium-volume range (max. $2m^3$)
  (Stirred systems in production processes for antibodies, and vaccines)

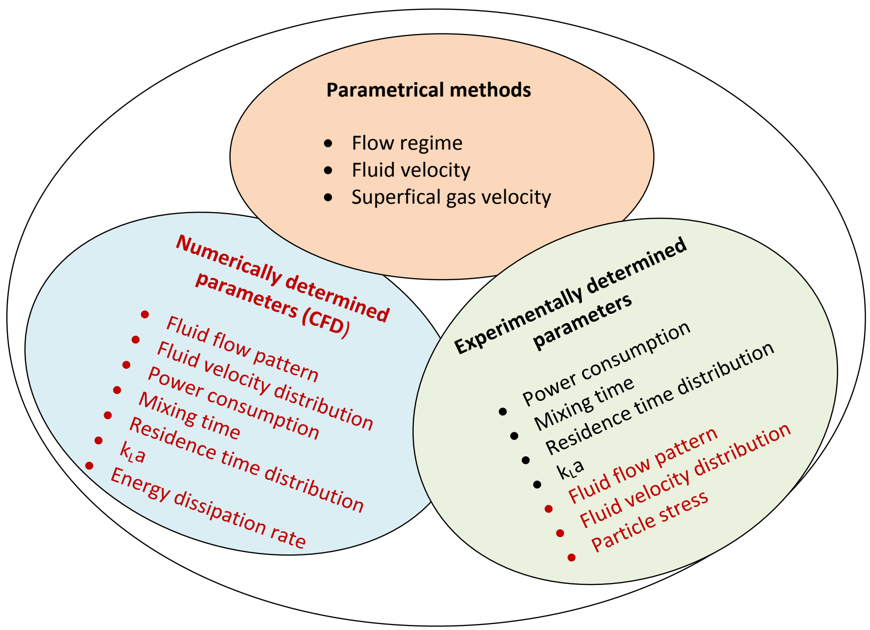
New applications and niche products

- Stirred and wave-mixed systems
  - Production of cell therapeutics based on stem- and T-cells
  - Development and production of plant cell-based bioactive compounds for cosmetic industry

Biochemical engineering characterization of SUB

Our approach for SUB applications

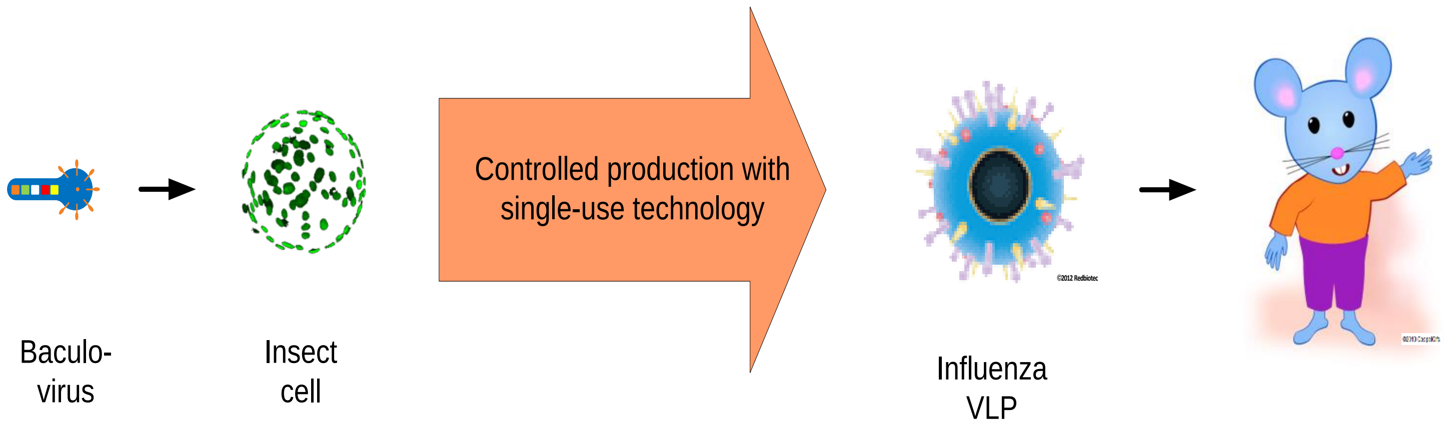
Better understanding and knowledge about mass and heat exchange, energy, pressure, velocity distributions in dependence of time and location in a SUB and the interactions of these parameters in order to speed up bioprocess developments and process scale-ups.



For more detailed information see C. Löffelholz et al. (2013)

Selected applications with SUBs

(ZHAW)



EXAMPLE 1: Wave-Mixed Bioreactors and Insect Cells

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Example 1: Aims

- Fast production process (max. 5 days)
- More efficient inoculum production
    - Perfusion for working cell bank generation
    - HCD working cell bank in cryobags
    - One-step inoculation and cell expansion
- Scalable up to 300 L working volume
- USP: Complete SU
- Two –phase production process
- Growth phase
- Production phase
    - Higher shear sensitivity
    - Higher 0_2 demands

Example 1: Bioengineering characterization

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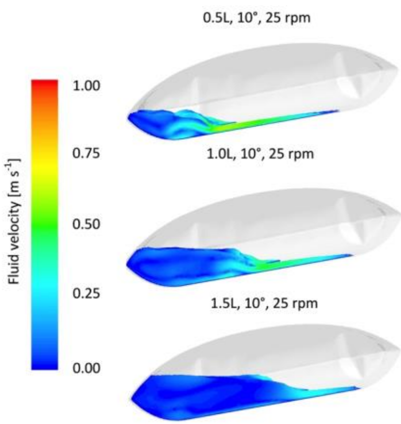
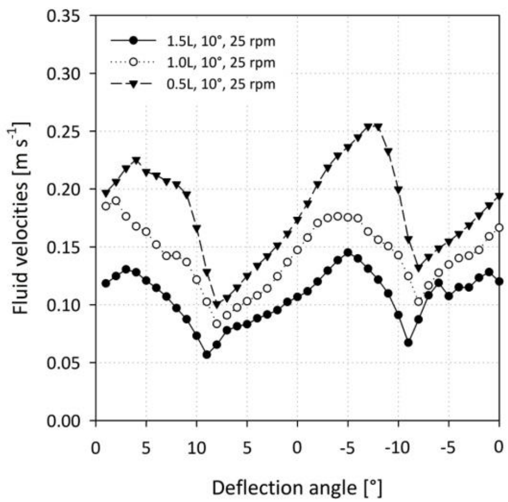
Fluid flow

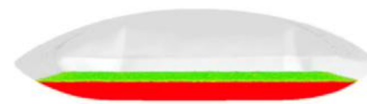


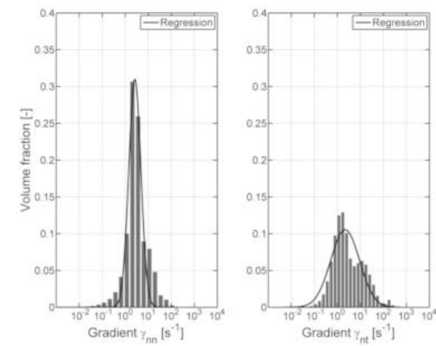


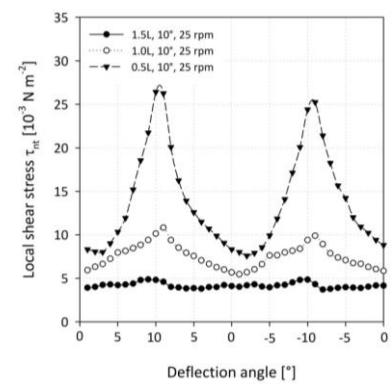
- Comparison of fluid flow during bag motion at different volumes
- CFD- predicted fluid velocities were validated by PIV-measurements (deviation < 5-10 %)

Hydrodynamic forces: shear stresses

(Framerate: 0.24 fps)



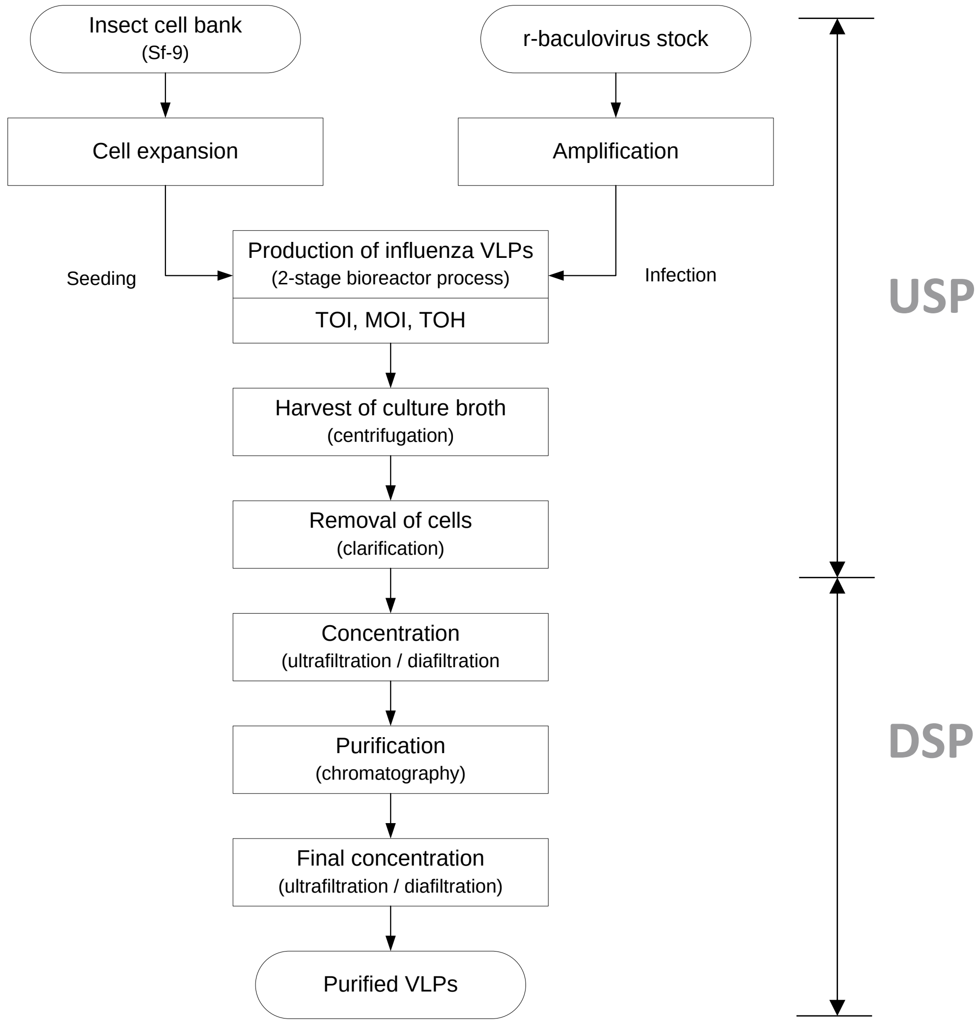




- Prediction of local normal and shear stress distribution during bag motion around one period
- Mean volume-weighted local shear stresses were compared between different volumes over momentary deflection angles.

For more detailed information see S. Werner et al. (2013) and Jossen et al. (2015)

Example 1: Process overview



For more detailed information see R. Eibl et al. (2014)

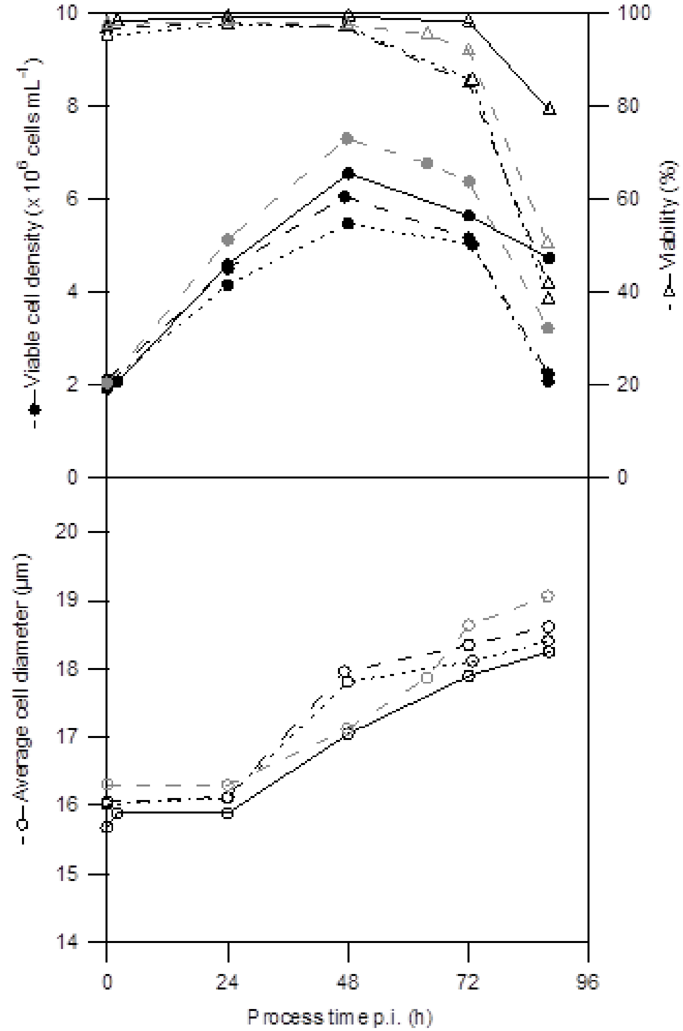
Example 1: Results

Preclinical sample productions: 2 L and 20 L Bags

- Cells from cryobags
- Virus from cryovials, V_2
- MOI > 0.01, TOI = $2*10^6$ cells mL^{-1}, TOH = 3-4 d.p.i.
  - Comparable growth/ infection kinetics

    D_{max} = $5.6\text{-}6*10^6$ cells mL^{-1} (2 d.p.i.)

    c_{HA} = 1-3 mg L^{-1}
- From inoculation via infection to harvest
  - 2 L: 6-9 d (2-5 d until infection)
  - 20 L: 8-11 d (via CultiBag 2 L, 4-7 d until infection)
- Scalable DSP (depth-filtration, TFF, IEX)
  - Purified VLPs
  - Functionality in HA-test demonstrated
  - Long-term storable at -80 °C

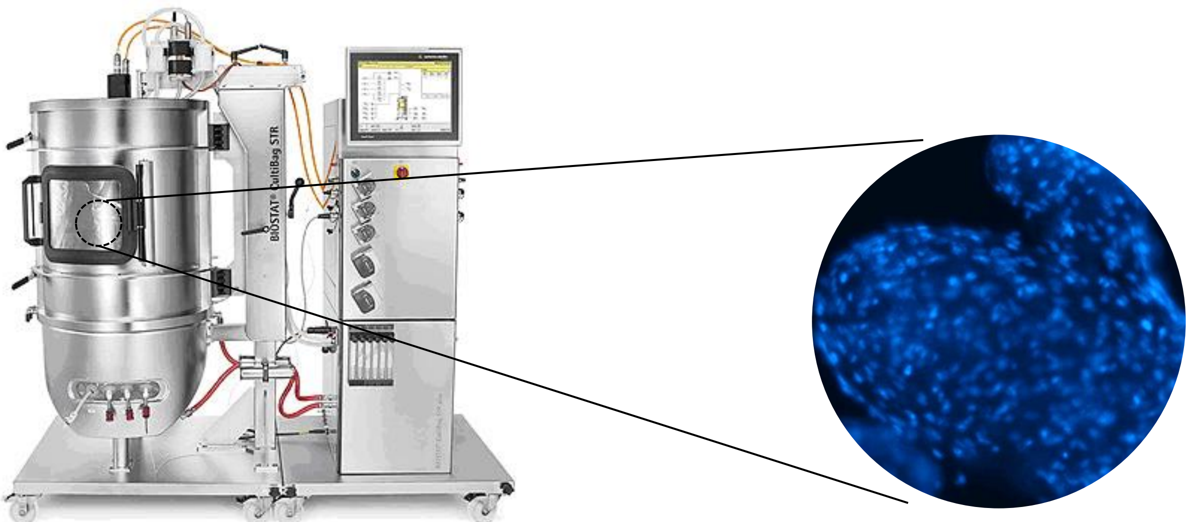


For more detailed information see R. Eibl et al. (2014)

Example 1: News

The challenge experiment using Redbiotec`s tetravalent influenza VLPs was successfully finalized. Results obtained clearly show that influenza VLPs are capable of stimulating the humoral and cellular immune responses which neutralize and inhibit replication of challenge virus. When immunized mice were challenged with a lethal dose of the parental virus, 100 % of mice were protected.

Schlieren, 10 May 2012



Soure: Sartorius Stedim Biotech

EXAMPLE 2: Microcarrier-Based Production of human mesenchymal stem cells in stirred single-use bioreactors (allogeneic therapies)

Example 2: Aims

- Scalable stirred process platform up to 50 L
- Cell yields of ≈ $1*10^{10}$ cells ($0.5*10^{6}$ cells mL^{-1})
- EFs > 30, 9 day cultivations
- No cell differentiation
- Two types of hMSCs (hADSCs and hBM-MSCs)
  - Provided by Lonza Cologne GmbH
  - Originated from a single donor
  - Cryopreserved, second passage, population doubling level of 10

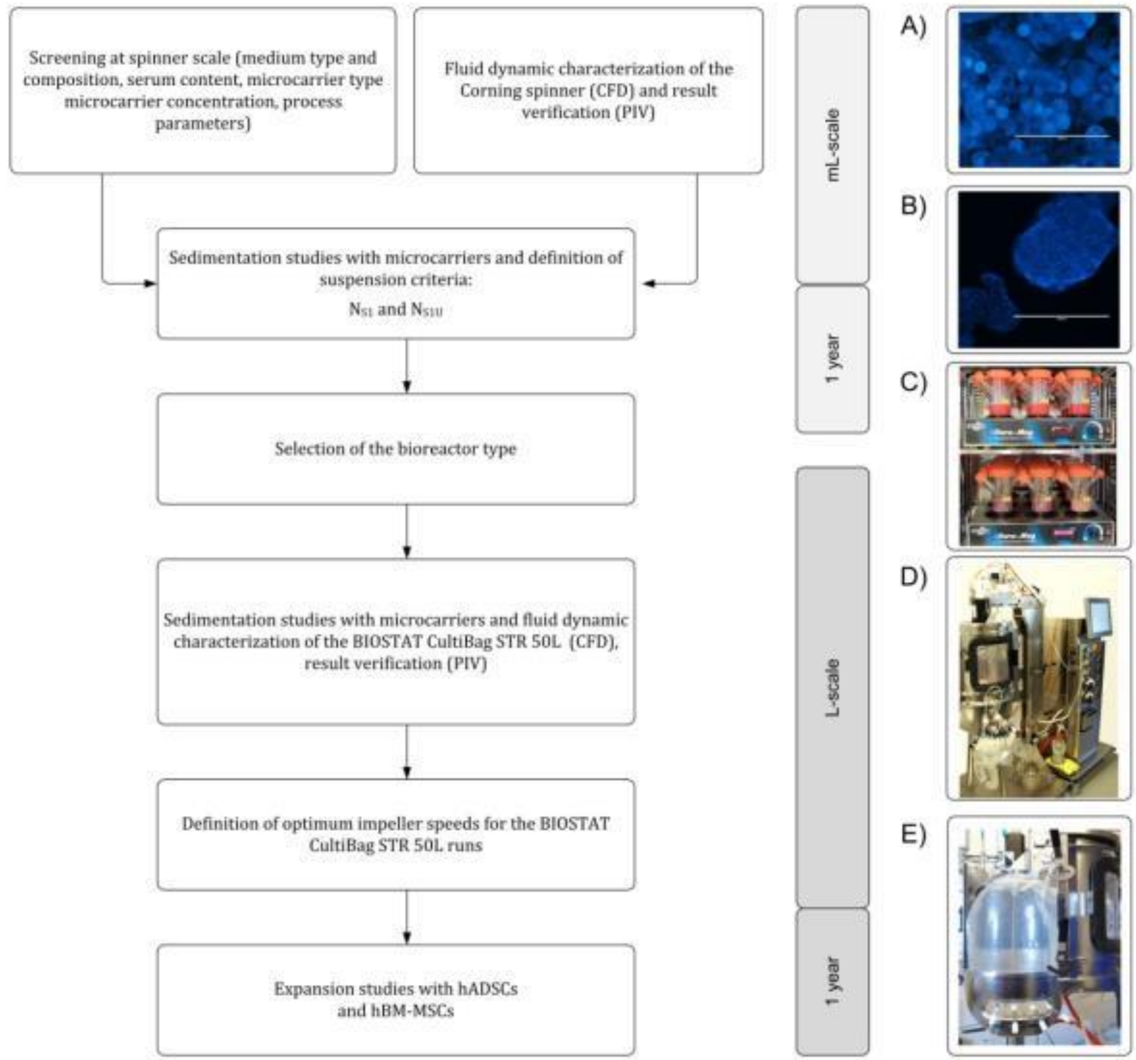
Example 2: Process overview

N_{S1}- criterion (just suspended criteria)

Impeller speed at which all particles are just fully suspended, whereby a homogeneous dispersion of all MCs is not a necessary consequence

N_{S1U}- criterion

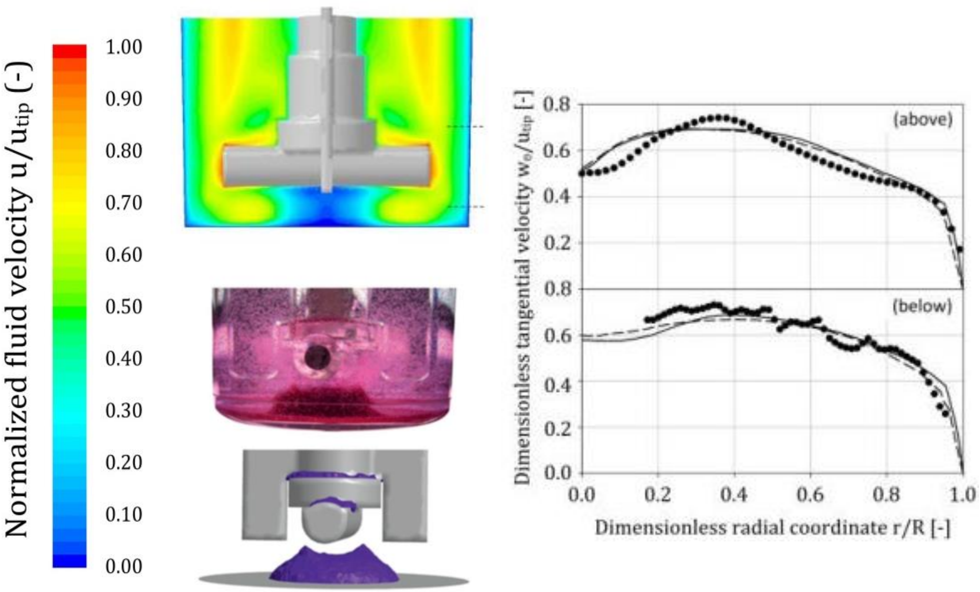
Impeller speed required to locate the MCs at the bottom of the bioreactor with none of them at rest



For more detailed information see C. Schirmaier et al. (2014) and V. Jossen et al. (2015)

Example 2: Biochemical engineering characterization

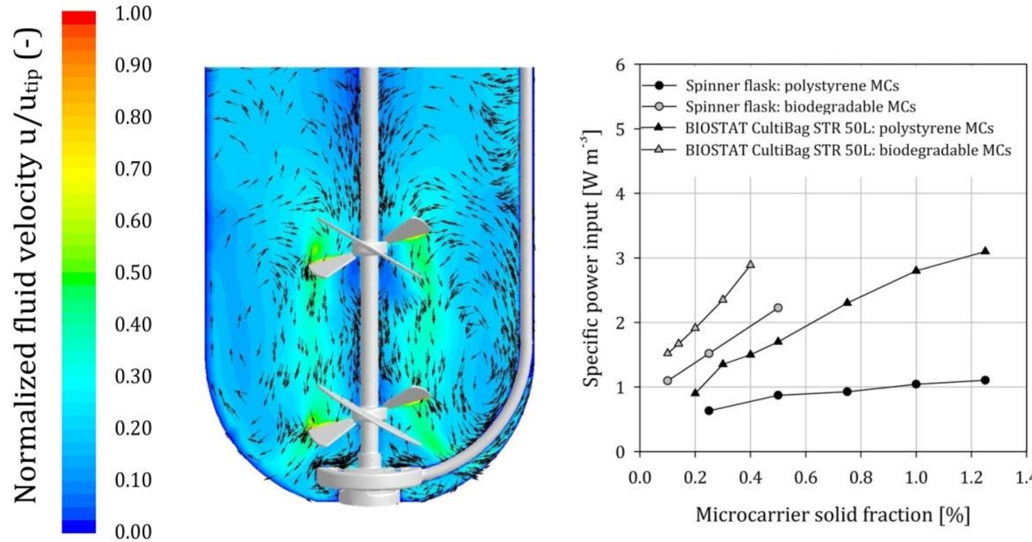
Corning spinner (mL-scale)



N < N_{S1U}

- Good agreement between the CFD- predicted and PIV-measured fluid velocities
- The model can be used to predict the MC- distribution
- Shear stress in the range of 0.004 to 0.2 Pa for hMSC cultivations

BIOSTAT STR 50L (pilot scale)

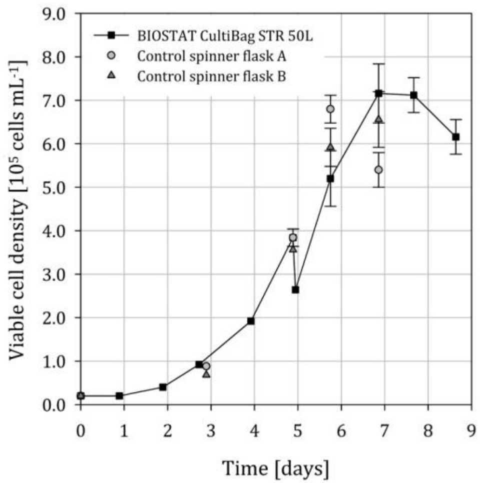


- Induced fluid flow is primary axial with two flow loops
- Axial velocities impinge on the reactor bottom
- Shear stress levels (not shown) comparable to spinner flasks

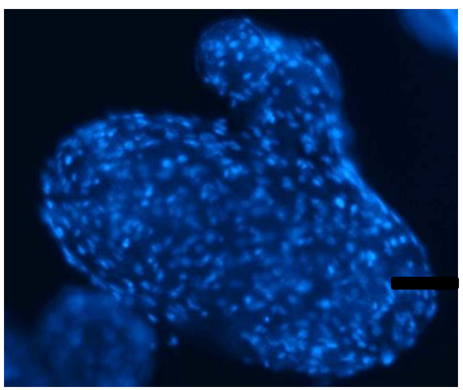
For more detailed information see St. C. Kaiser et al. (2013) and Jossen et al. (2015)

Example 2: Results

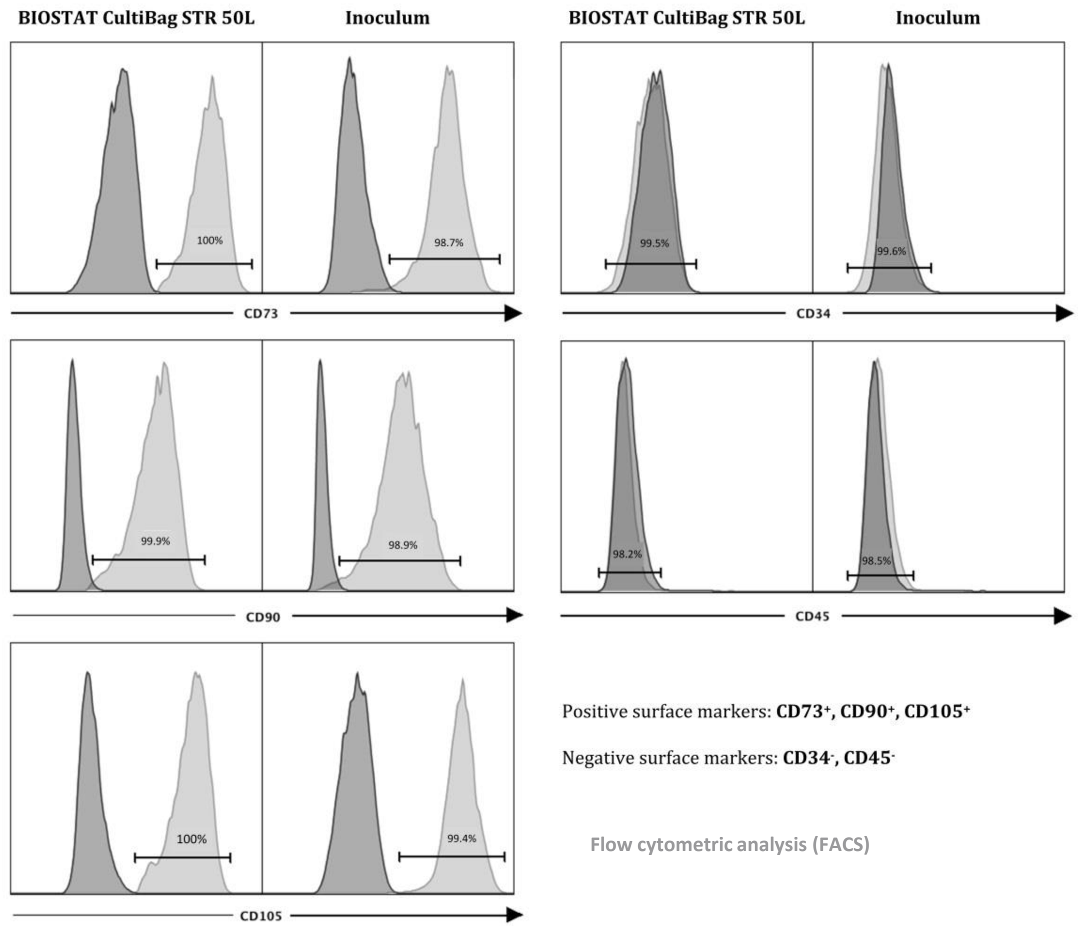
BIOSTAT STR 50 L-based expansion of hMSCs (Peak yields of $1 \cdot 10^{10}$ hADSCs (35 L) and $3.6 \cdot 10^{10}$ hBM-MSCs (50 L))



Optimized hBM-MSC cultivation at 50L scale (feed on day 5)



DAPI staining of hBM-MSCs on microcarrier surface.

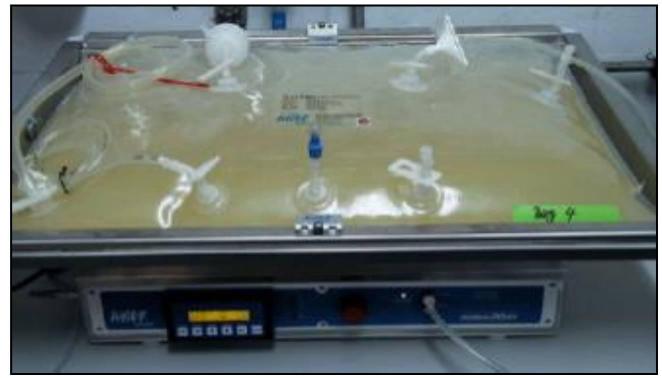


Positive surface markers: **CD73⁺, CD90⁺, CD105⁺**

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

Flow cytometric analysis (FACS)

For more detailed information see and V. Jossen et al. (2014b)



EXAMPLE 3: Wave-mixed Bioreactors and Plant Suspension Cells (PhytoCellTec^{TM} Malus domestica)

Example 3: Aims

- Robust batch production process for apple suspension cells
  - Cultivar Uttwiler Spätlauber (Swiss apple)
  - 1 L, 10 L and 25 L working volume
  - Cultivation time up to 25 days
  - Heterotrophic culture (no light)
- High Biomass fresh weight
  - 30 kg per 25 L working volume
  - Doubling time between 2 and 4 days
- Bioactive biomass ingredients
  - Extract as cosmetic active ingredient in different cosmetic brands worldwide (antiaging)

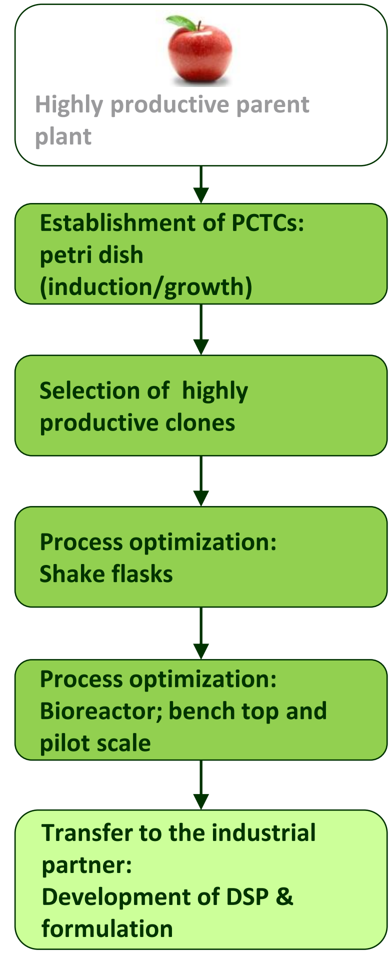
Example 3: Generation of apple suspension cells and their mass propagation

Establishment of callus cultures from apple core

- Surface sterilization
- Callus induction and maintenance
- Friable, well-growing and bioactive callus → High viability

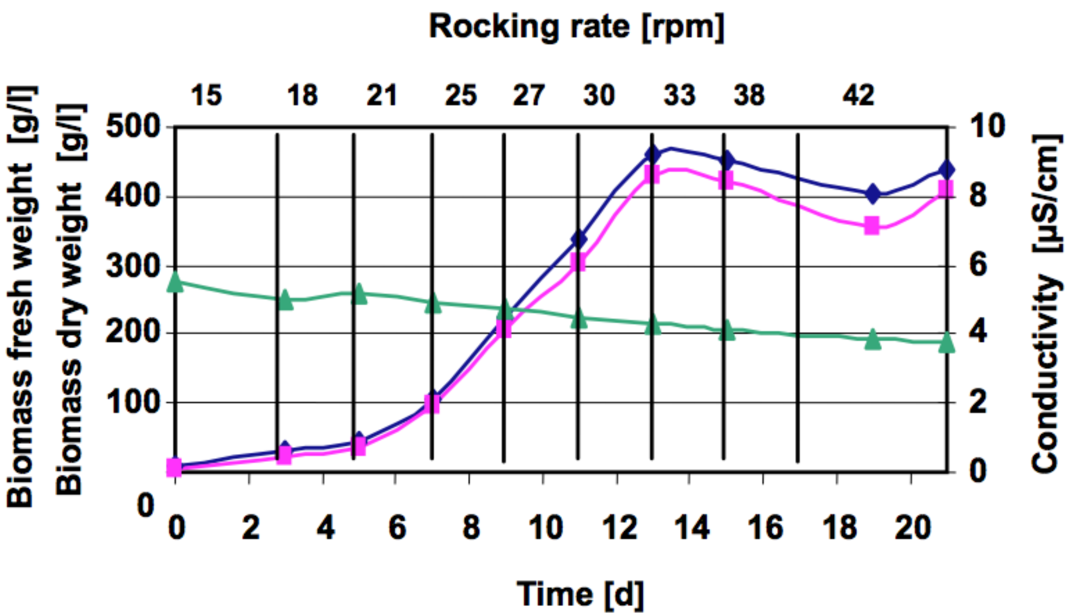
Establishment of a well-growing and bioactive suspension culture in shake flasks

- Induction via homogenization
- Maintenance cultivation and long-term storage of suspension cells
- Optimization of mass propagation in shake flasks
- Process transfer to a wave-mixed bioreactor
  - ➢ Problems: Aggregation of plant cells, increasing viscosity and change of the fluid flow behaviour of the culture broth → Possible mass transfer limitations

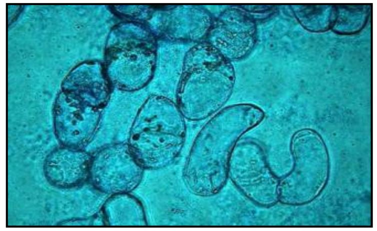


For more detailed information see and N. Imseng et al. (2014)

Example 3: Results of 2 L and 20 L CultiBag



Growth of the apple suspension cells in the BIOSTAT CultiBag RM



- High pressure homogenization of the biomass and incorporation of the extract into lecithin
- Main ingredients: Xanthan, gum, glycerin, lecithin, phenoxyethanol, aqua

For more detailed information see and N. Imseng et al. (2014)

Successful product launch and further plant cell-based products from wave-mixed SUBs

PhytoCellTec Alp Rose

PhytoCellTec Argan

PhytoCellTec Solar Vitis

PhytoCellTec Symphytum

PhytoCellTec nunatak

http://www.lifepr.de/pressemitteilung/med-beauty-swiss-ag/cell-premium-icon-by-Dr-Harald-Gerny-Jugendliche-Haut-Ein-Leben-lang/boxid/365416 7/2015,
Mibelle Biochemie AG /7/2014

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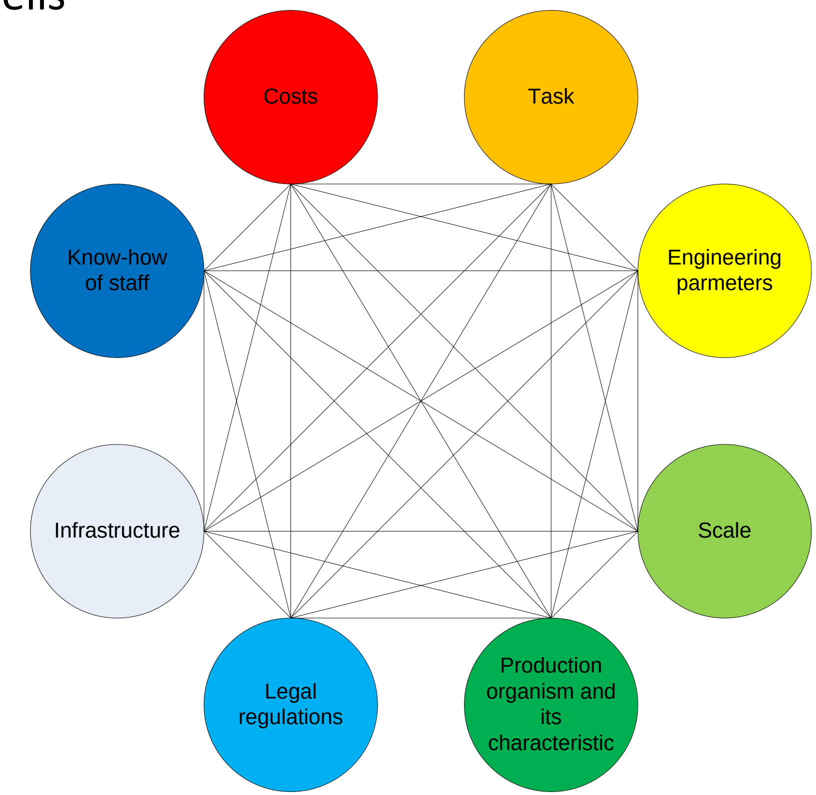
Summary

- Single-Use bioreactors are well established although they are not always optimal
- Can be used for the cultivation of different types of cells (e.g. insect cells, stem cells, plant cells, …)
- The production processes in SUB's are scalable
  - Geometrically similar and non-similar systems
- Biochemical engineering characterization important
  - Characterization guidelines (DECHEMA 2016)



Acknowledgments

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Thank you!