PerfuseCell

Hot subject to present

Perfusion Single-Use-Bioreactor integrating both Single-Use-Pump and single-use cell retention Cross-Flow-Filter

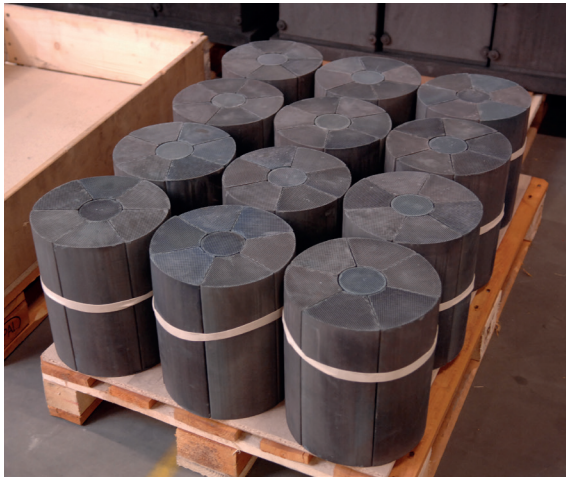
Per Stobbe

Presentation as of June 27th 2016

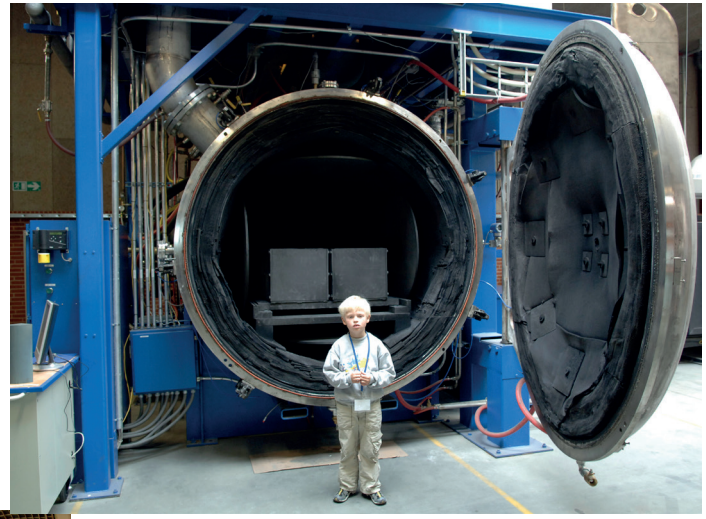
Stobbe history

Porous materials, fluid dynamics, material science, processes

Development of products, processes, IPR, companies



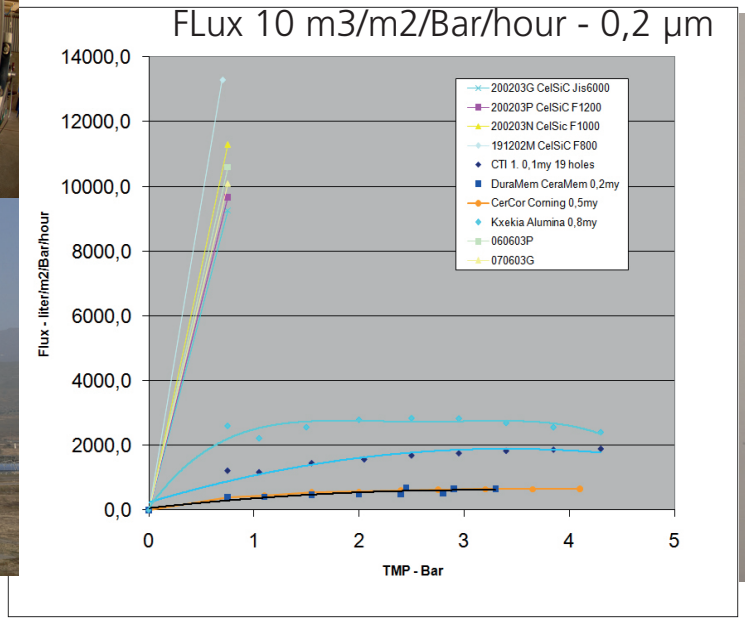
Hot gas filters



Hot process at 2.500°C

Nano particle separation

Hot surfaces



Stobbe Group structure

R&D company from 1986 - mother of five - **www.stobbe.com**

R&D company from 2015 - farther to all - **www.stobbepharma.ch**

CerCell

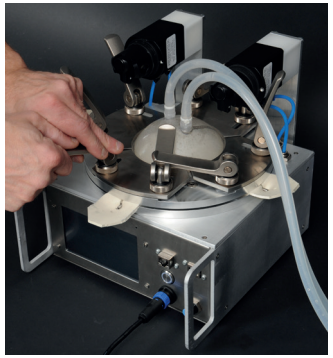
Configurable Single-Use products for cultivation and fermentation of cells in suspension and on beads.

www.cercell.com

PumpCell

Single-Use-Pump
Fully programmable
5 bar, few mL/hour to 50 liter/min, no sheading.

www.pumpcell.com



PerfuseCell

Cell retention Perfusion-Single-Use-Bioreactor for continuous expression of anti-bodies.

www.perfusecell.com

Cronus-PCS

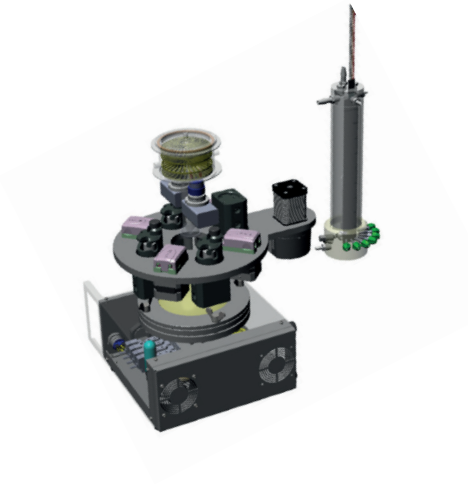
Configurable Process-Control-System, open platform with 30 different active components, software and Single-Use-Sensors.

www.cronus-pcs.com

ProlifeCell

Perfusion-Single-Use-Bioreactor harbouring cells for continuous proliferation of stem cells in scalable platform.

www.prolifecell.com



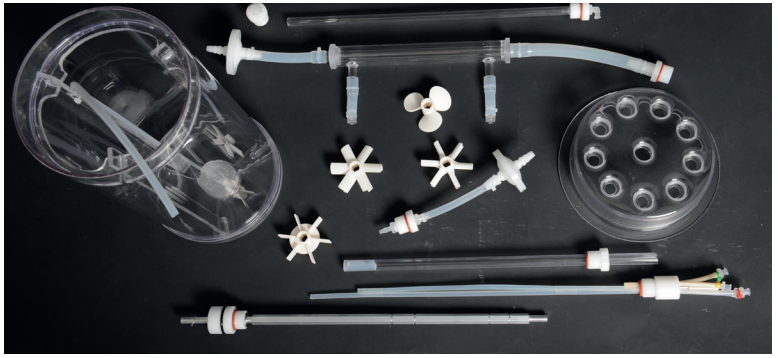
Presentation as of June 27th 2016

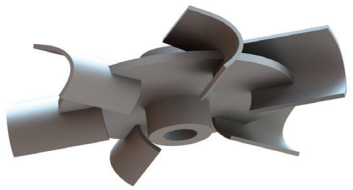
CerCell

Configurable Single-Use products for cultivation and fermentation of cells in suspension and on beads.

www.cercell.com

The "Configurator Tool" is a world first and only "Do-It-Yourself" SUB and SUF product. You can choose from +5.000 different components on the shelfs at CerCell.





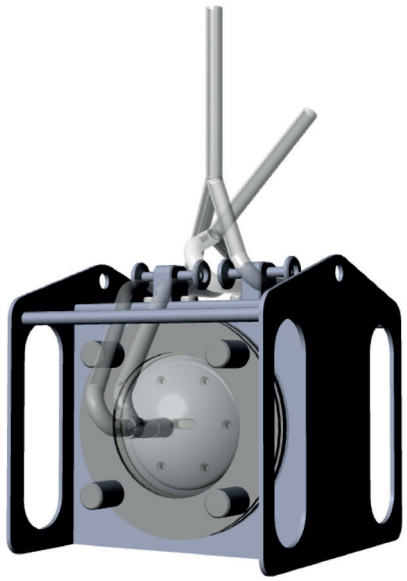
Presentation as of June 27th 2016

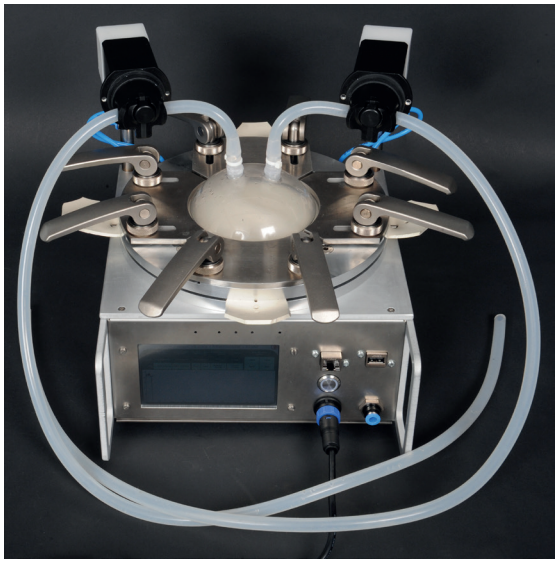
PumpCell

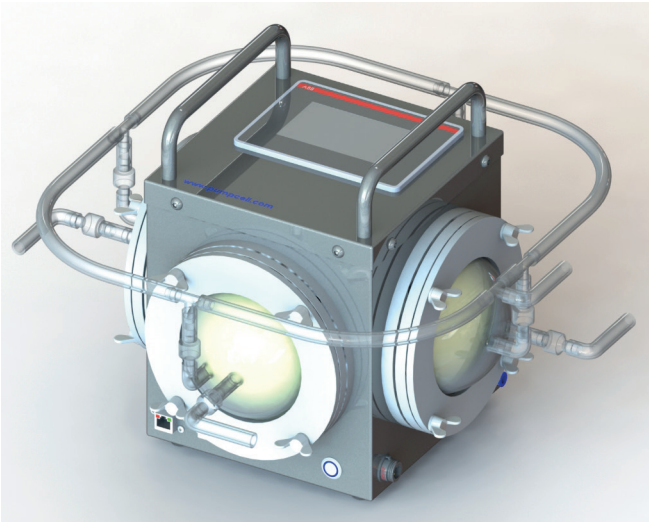
The Mnemosyne family is world first CPU controlled free-flowing diaphragm pump. Simple and low cost Single-Use parts as easy to replace as a hose. No shedding of nano-size silicone particles. Positive displament pump which accurately measures pumped volume.

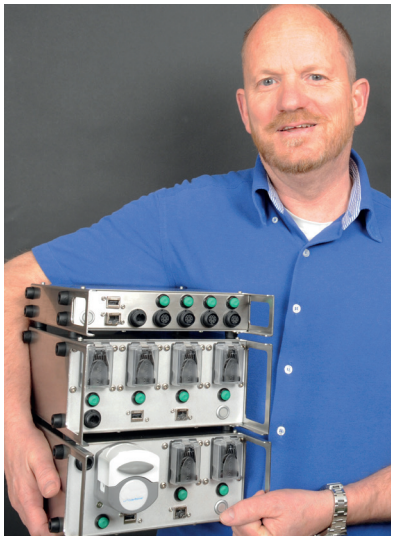
Single-Use-Pump
Fully programmable functions
5 bar pressure, few mL/hour to 50 liter/min
No nano-particles in fluid stream

www.pumpcell.com









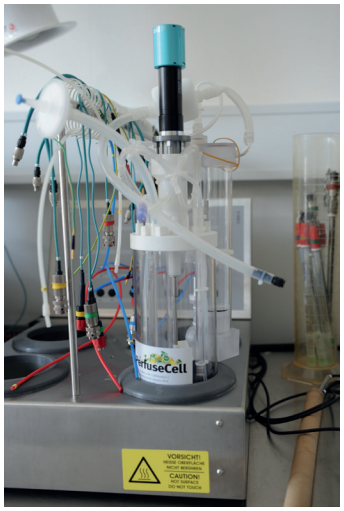
Presentation as of June 27th 2016

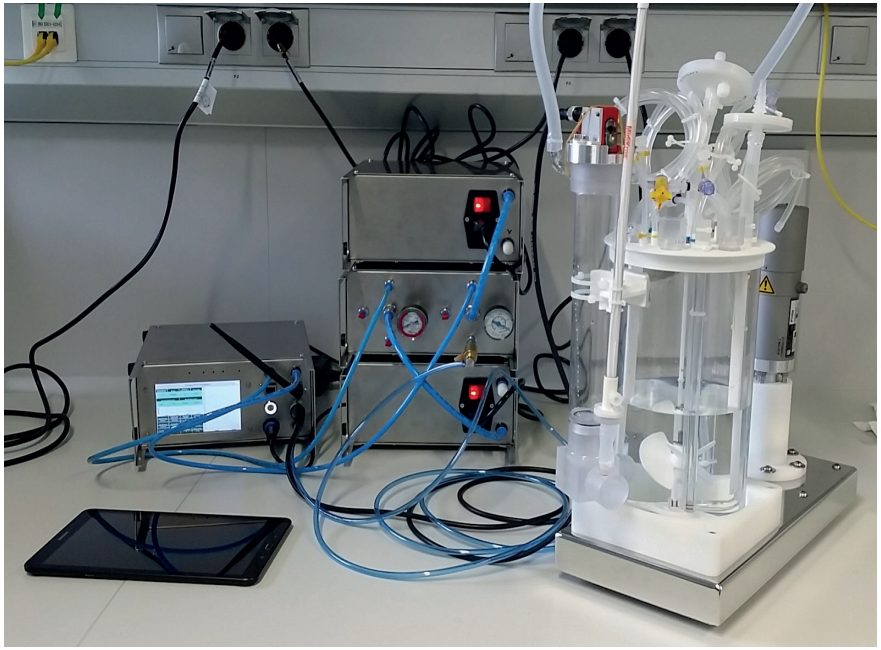
PerfuseCell

Customised **Perfusion-Single-Use-Bioreactor** for expressing a product during cultivation from as much as 150 mio/mammalian cells/ml. Micro-carriers, macro-carriers, scaffold in a packed-bed.

Cell retention Perfusion-Single-Use-Bioreactor for continuous expression of anti-bodies.

www.perfusecell.com





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Very high cell density perfusion of CHO cells anchored in a non-woven matrix-based bioreactor

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IgG production
Dielectric spectroscopy
Hypothermia

ABSTRACT

Recombinant Chinese Hamster Ovary (CHO) cells producing IgG monoclonal antibody were cultivated in a novel perfusion culture system CellTank, integrating the bioreactor and the cell retention function. In this system, the cells were harbored in a non-woven polyester matrix perfused by the culture medium and immersed in a reservoir. Although adapted to suspension, the CHO cells stayed entrapped in the matrix. The cell-free medium was efficiently circulated from the reservoir into- and through the matrix by a centrifugal pump placed at the bottom of the bioreactor resulting in highly homogenous concentrations of the nutrients and metabolites in the whole system as confirmed by measurements from different sampling locations. A real-time biomass sensor using the dielectric properties of living cells was used to measure the cell density. The performances of the CellTank were studied in three perfusion runs. A very high cell density measured as 200 pF/cm (where 1 pF/cm is equivalent to 1×10^6 viable cells/mL) was achieved at a perfusion rate of 10 reactor volumes per day (RV/day) in the first run. In the second run, the effect of cell growth arrest by hypothermia at temperatures lowered gradually from 37 °C to 29 °C was studied during 13 days at cell densities above 100 pF/cm. Finally a production run was performed at high cell densities, where a temperature shift to 31 °C was applied at cell density 100 pF/cm during a production period of 14 days in minimized feeding conditions. The IgG concentrations were comparable in the matrix and in the harvest line in all the runs, indicating no retention of the product of interest. The cell specific productivity was comparable or higher than in Erlenmeyer flask batch culture. During the production run, the final harvested IgG production was 35 times higher in the CellTank compared to a repeated batch culture in the same vessel volume during the same time period.

1. Introduction

Perfusion bioprocesses have several advantages compared with batch/fed-batch processes such as a potential high cell density, a high productivity in a relatively small size bioreactor, a stable cell environment and long-term production (Castilho and Medronho, 2002; Chotteau, 2015; Chu and Robinson, 2001; Langer, 2011; Voisard et al., 2003).

Perfusion processes have become increasingly accepted in the past decade for the commercial manufacturing of biopharmaceuticals due to, on one hand, the increasing use of disposable bioreactor systems alleviating the technical and sterility challenges, the need of flexibility and smaller equipment footprint for manufacturing, and, on the other hand, the emergence of robust perfusion systems, e.g., the alternating tangential flow filtration (Clincke et al., 2013a,b). Another field of application of perfusion processes is the production of biologics as research tools where given amounts of protein products are needed within a very short time period. For this kind of application, quite often neither the cell line nor the process/cultivation medium is optimized. High cell densities potentially provided by the perfusion mode can advantageously compensate for these sub-optimal conditions. Furthermore, perfusion mode offers a stable and continuously renewed cell environment favorable for the control of the product quality and can be necessary in the case of labile proteins.

Abbreviations: CHO, Chinese Hamster Ovary; CSPR, cell specific perfusion rate; DO, dissolved oxygen; HPLC, high-performance liquid chromatography; LDH, lactate dehydrogenase; RV/day, reactor volume per day.

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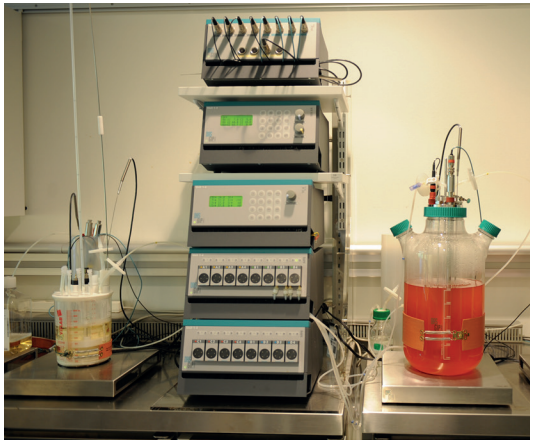
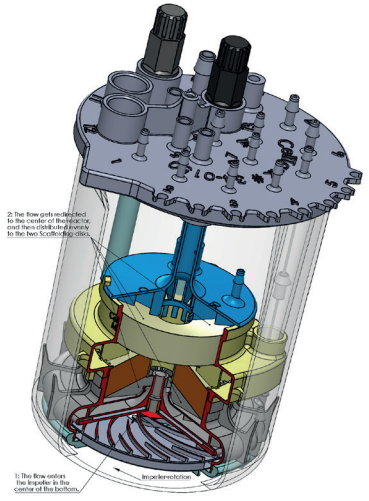
[1] Presently Serendipity Innovations, Sturegatan 15, SE-11145 Stockholm, Sweden.

http://dx.doi.org/10.1016/j.jbiotec.2015.07.006
0168-1656/





Presentation as of June 27th 2016

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Configurable Process-Control-System, open platform with 30 different active components, software and Single-Use-Sensors.

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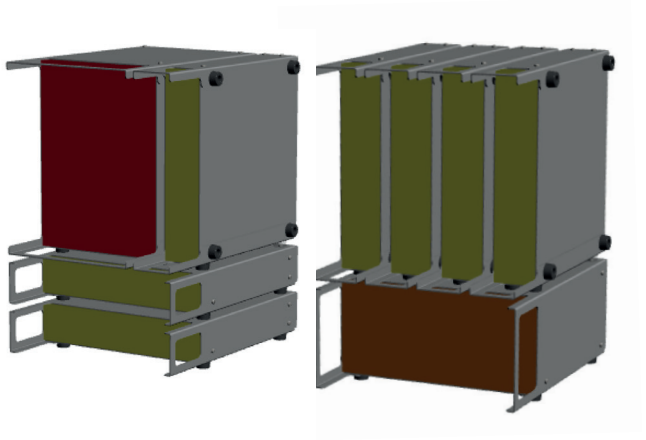
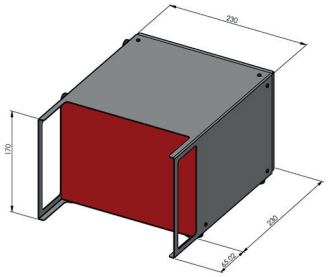
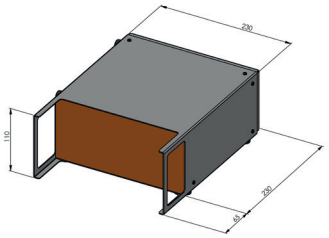
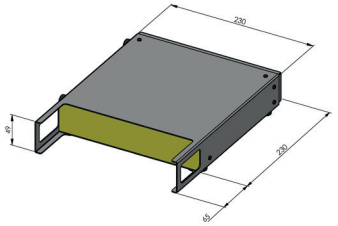








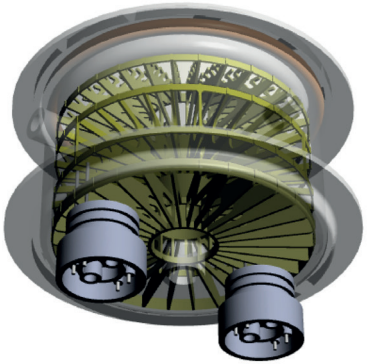
Presentation as of June 27th 2016

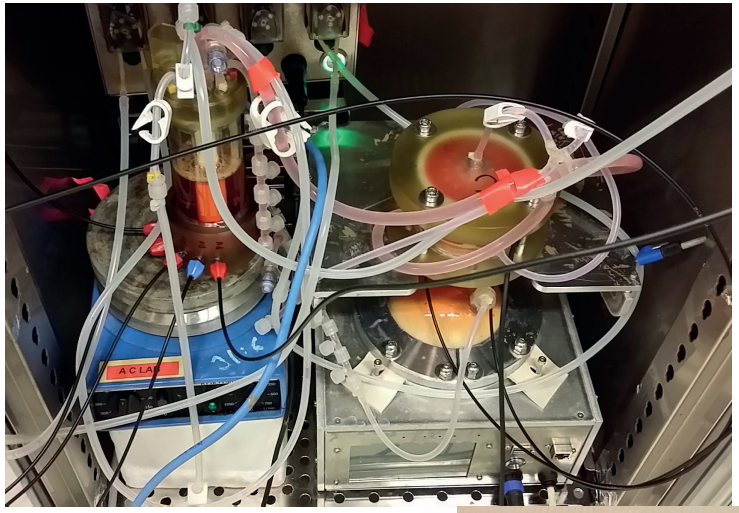
ProlifeCell

Perfusion-Single-Use-Bioreactor harbouring cells for continuous proliferation of stem cells in scalable platform.

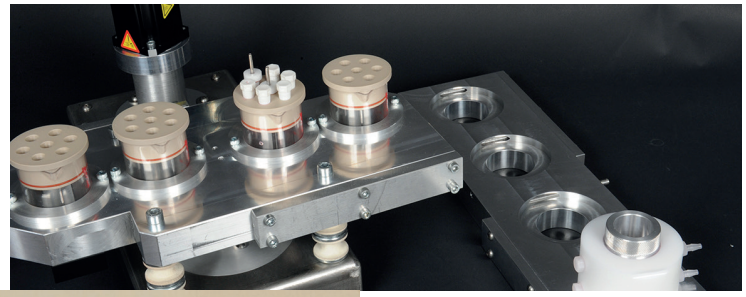
www.prolifecell.com

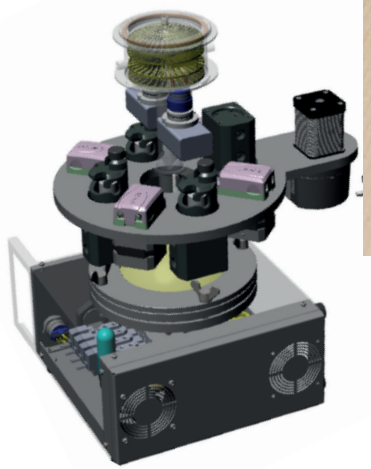
Customised cutting-edge Perfusion-Single-Use-Bioreactor (P-SUB) platform for **proliferation of stem cells** in suspension or integrating micro-carriers, macro-carriers, porous scaffold in a packed-bed. Combined with Mnemosyne diaphragm pump for all fluid transfer and harvest.

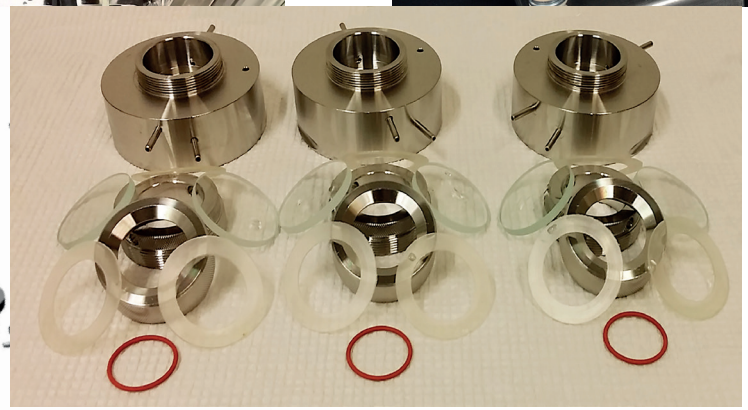


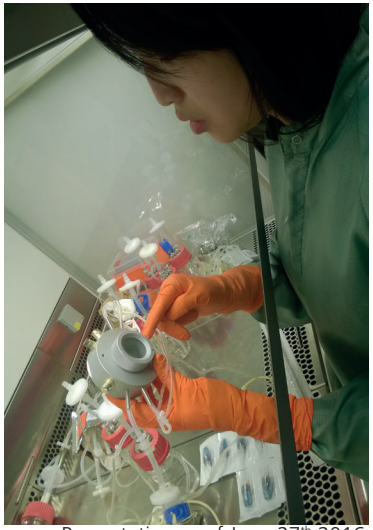


50 ml scaffold volume on top of Mnemosyne pump









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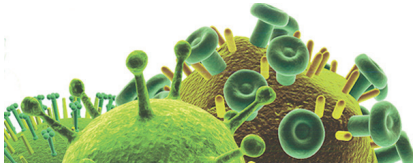
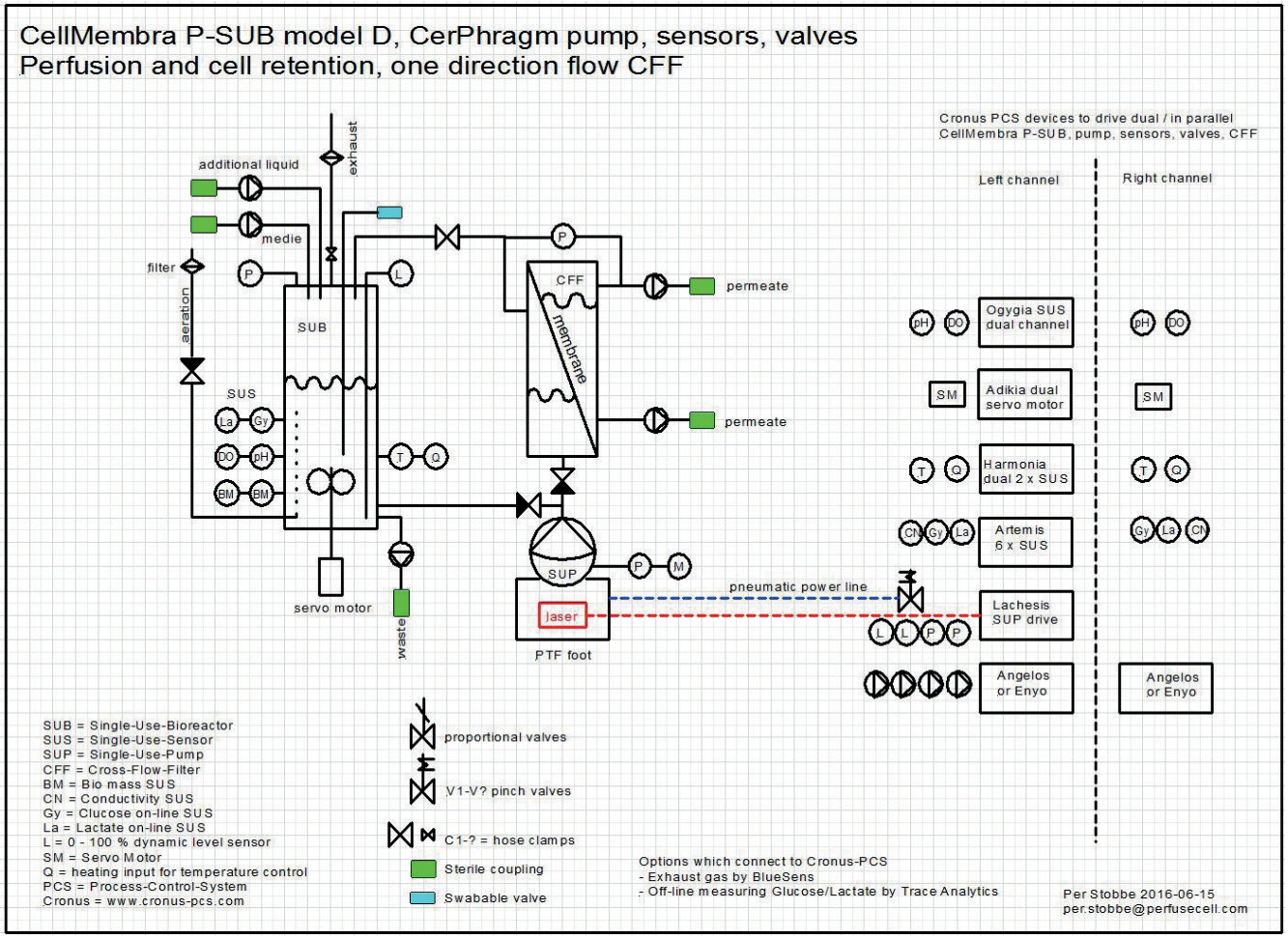


Cell retention with membranes

Subject to tumble:
“Perfusion Single-Use-Bioreactor integrating both Single-Use-Pump and single-use cell retention Cross-Flow-Filter”



Pulsating-Tangential-Flow is the new concept - **PTF**

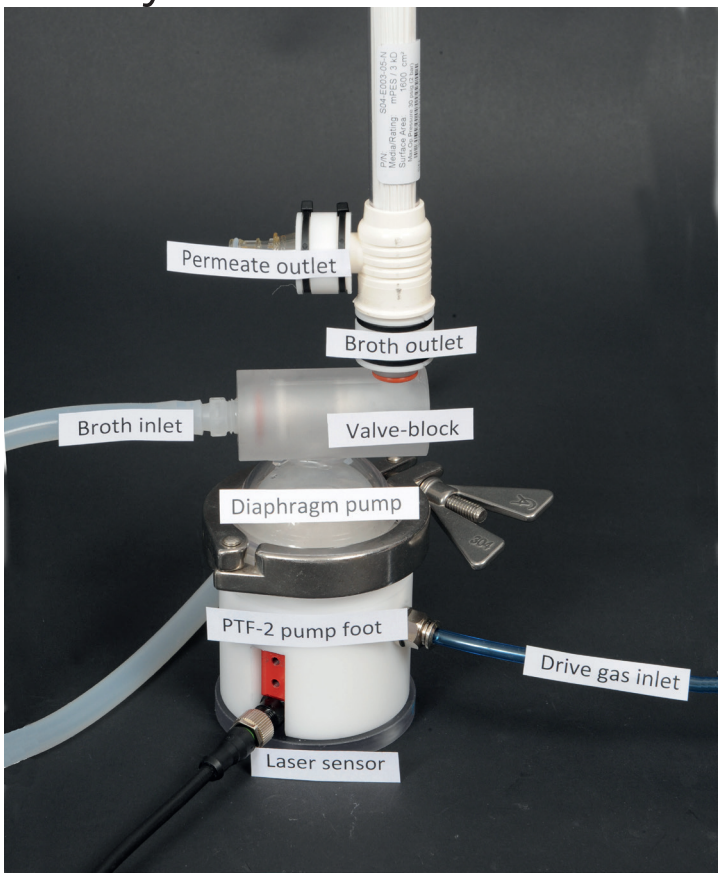
The pump and valve-block creates the difference

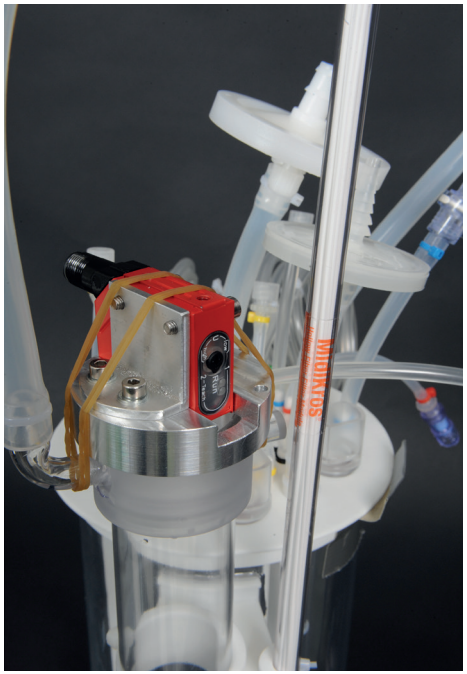
Presentation as of June 27th 2016

Cell retention with membranes

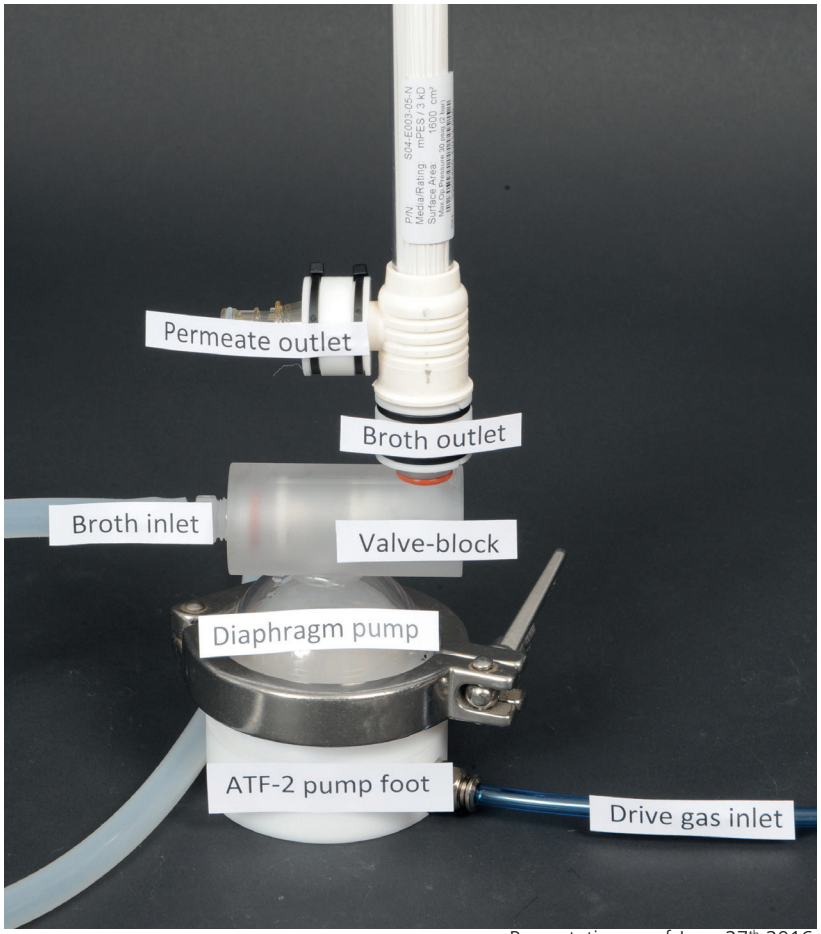
Which is the secret ?

The secret is to know where the diaphragm is at any time





Laser sensor is the secret of the new PTF concept



Cell retention with membranes

Very small size P-SUB

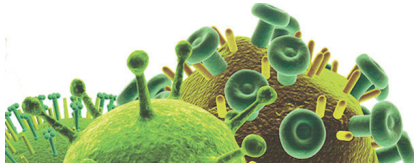
Perhaps world smallest?

The bioBLU® P-SUB fits into the DASbox®

Run 16 P-SUBs in parallel

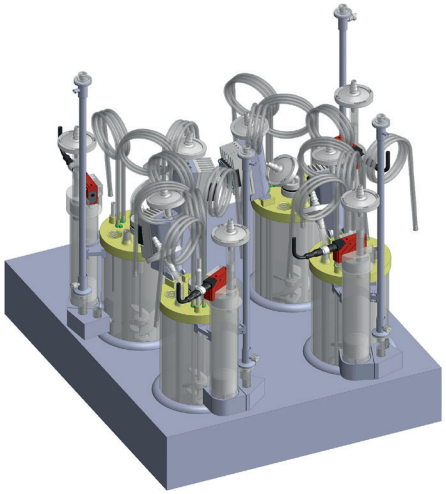
500 ml
Vessel Volume

100 - 400 ml
Working Volume

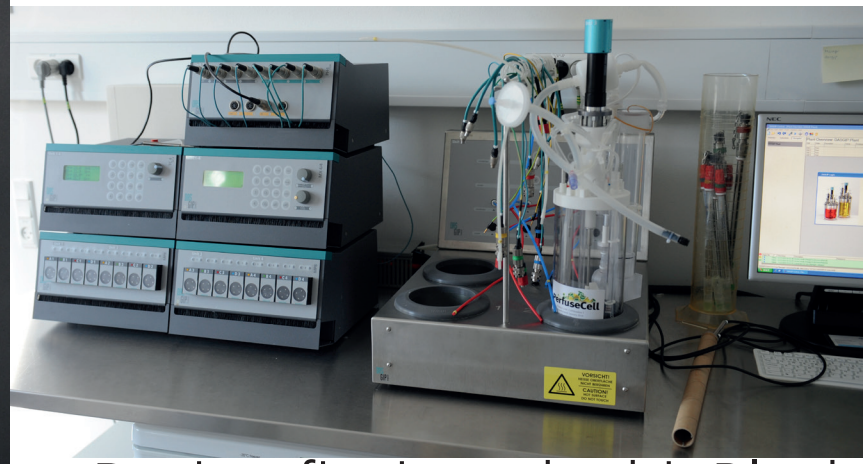


Small size / 2 litre
P-SUB-C

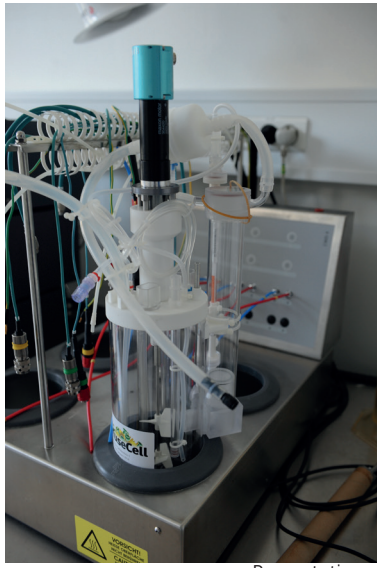
- Top drive
- Bottom drive



Cell retention with membranes



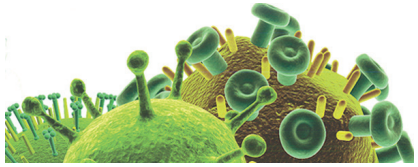
Design fits into the bioBlock



Cell retention with membranes

Medium size P-SUB-C

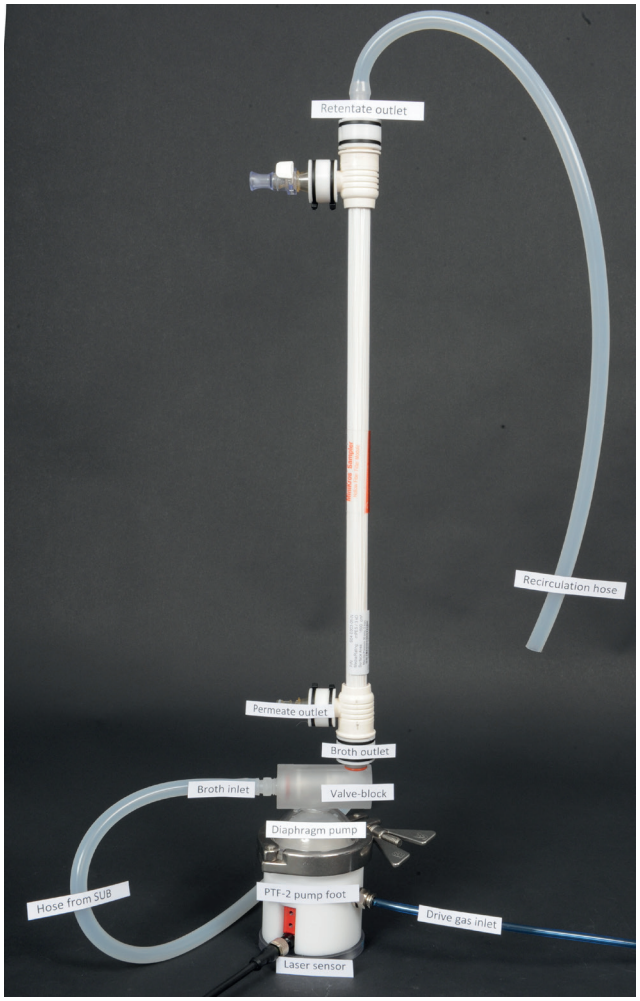
- 3 liter VV
- Fully customizable
- Fully scalable
- Any CFF
- Top drive
- Bottom drive



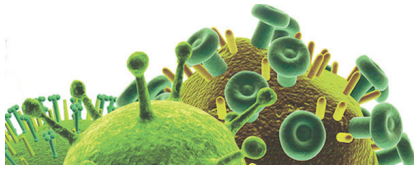
Medium size P-SUB-D

- 3 liter VV
- Fully customizable
- Fully scalable

Cell retention with membranes

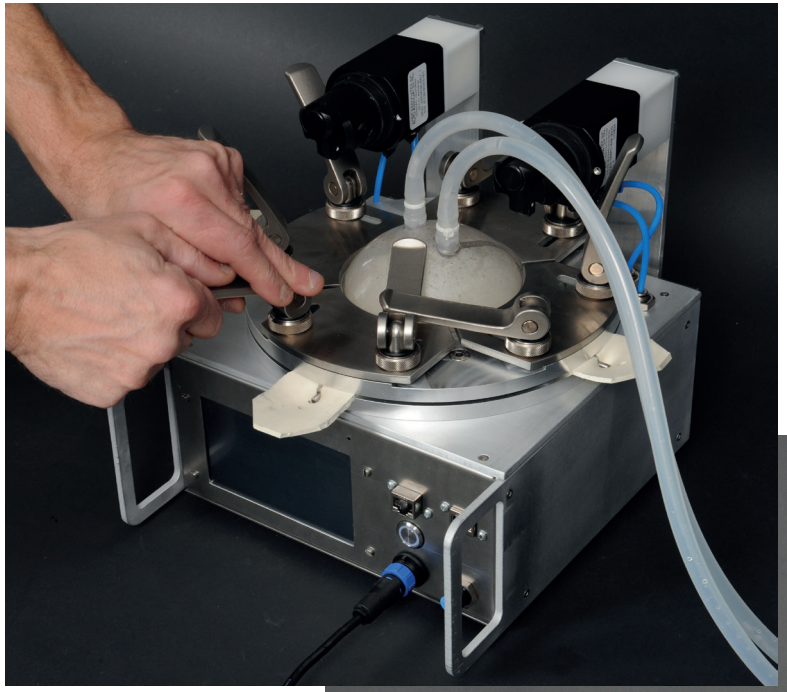


World first Perfusion-Single-Use-Bioreactor (P-SUB)!

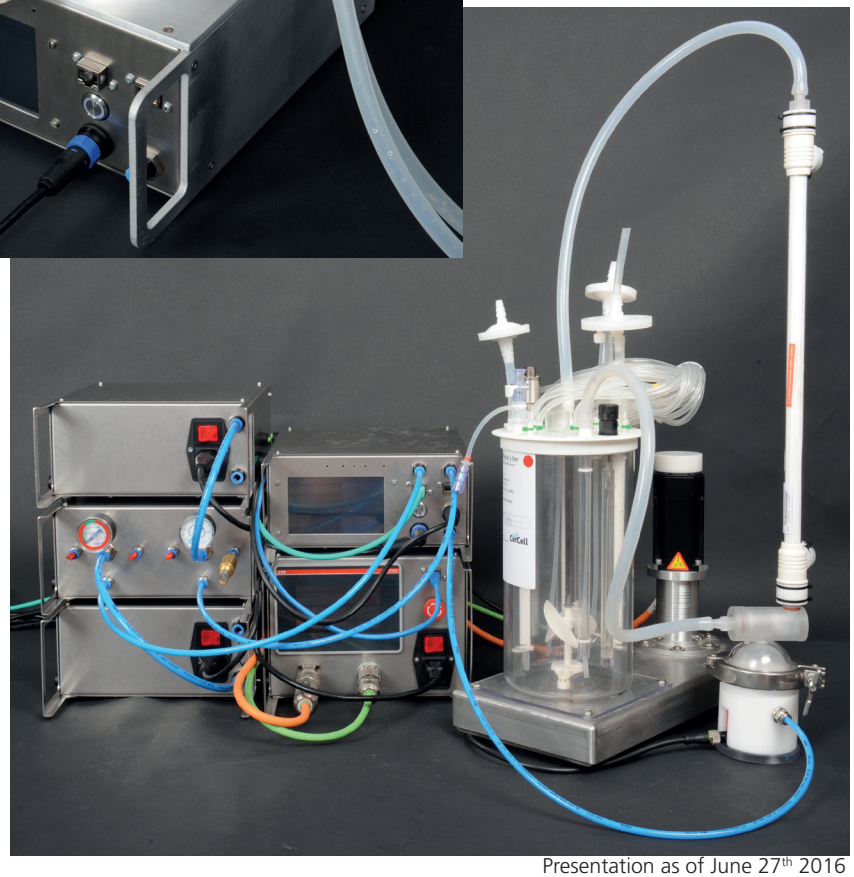


Cell retention with membranes

CPU control of P-SUB pump either air-Column or Diaphragm. The Laser sensor measure with 0,1 mm accuracy the actual position of the diaphragm. From here a CPU can be programmed to perform any desired cycle.



P-SUB preferably controlled by the Lachesis unit receiving vacuum and pressure from Sarpedon and Alagonia unit. Magnetic-Stirrer-Table driven by the Horae unit.



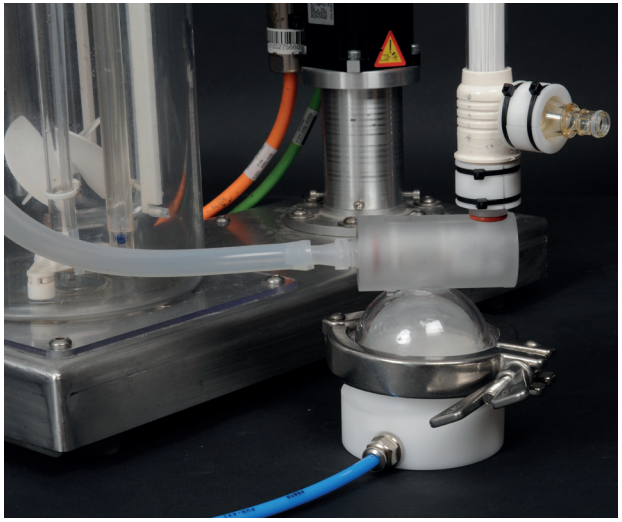
The pump product we call Mnemosyne is based on our issued EP patent and US patent application - we don´t infringe!

Cell retention with membranes

CellMembra P-SUB-D even work on ATF-2 pump foot with ATF controller.

CellMembra do not infringe ATF IPR owned by Repligen.

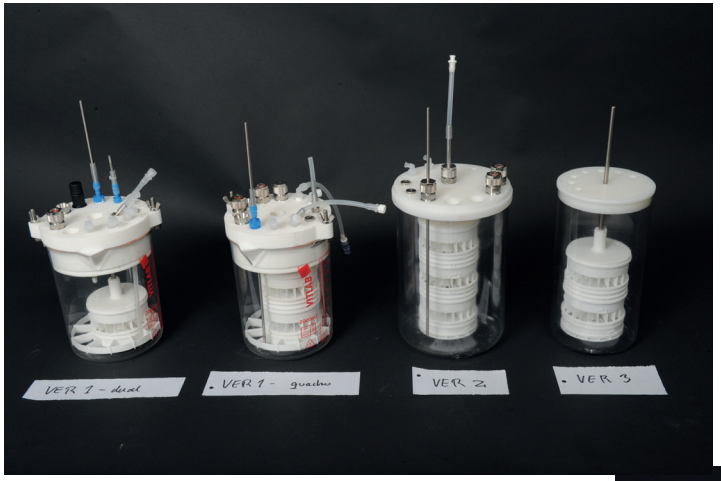
A direct replacement



Valve-block insure broth flow in one direction through CFF

Cell retention with depth filters

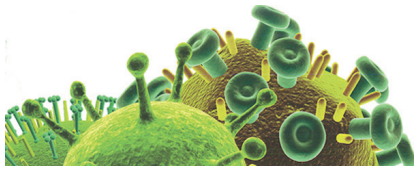
CellCore history



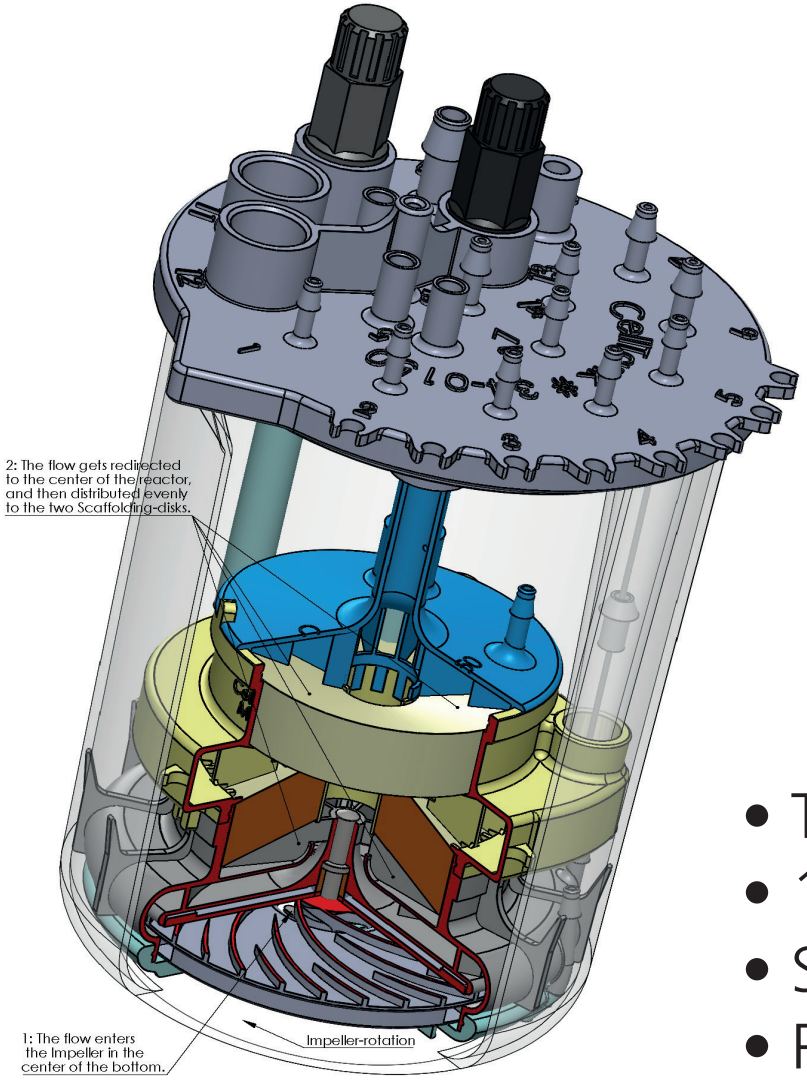
CellCore is the concept where porosity, pore size, fibre diameter, surface properties is balanced out in a packed bed.

CellCore is scalable independent of the packing material

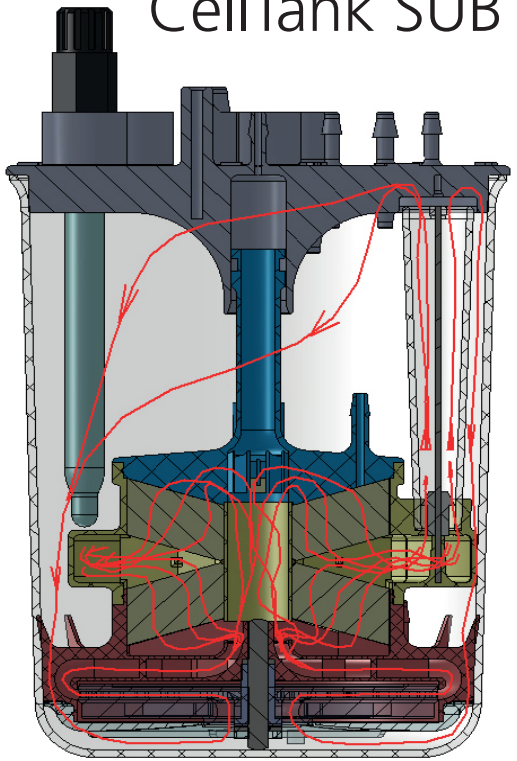
Steady-state continuous processing require cells are exposed for constant condition. Not really what we see in ATF or PTF perfusion mode!



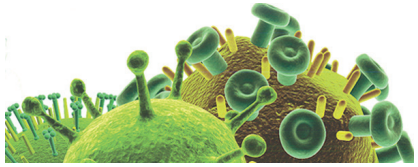
Cell´s harboured in depth filters



CellTank SUB



- True steady-state conditions
- 150-200 mio/ml suspension or adherent cells
- Scalable from 15 to 15.000 ml scaffold
- Packed bed with fibrous scaffold
- Good results with centrifugal pumps



Cell´s harboured in depth filters

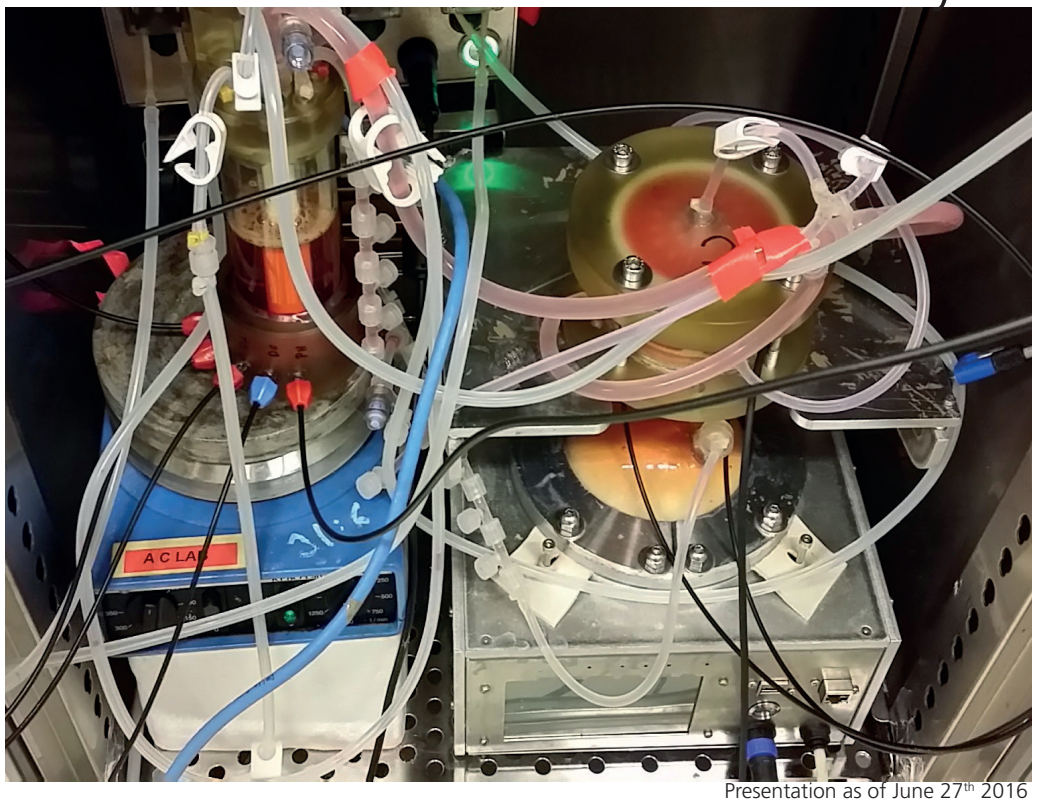
- True steady-state conditions
- 150-200 mio/ml suspension or adherent cells
- Scalable from 15 to 15.000 ml scaffold
- In test phase with diaphragm pump

HESUB

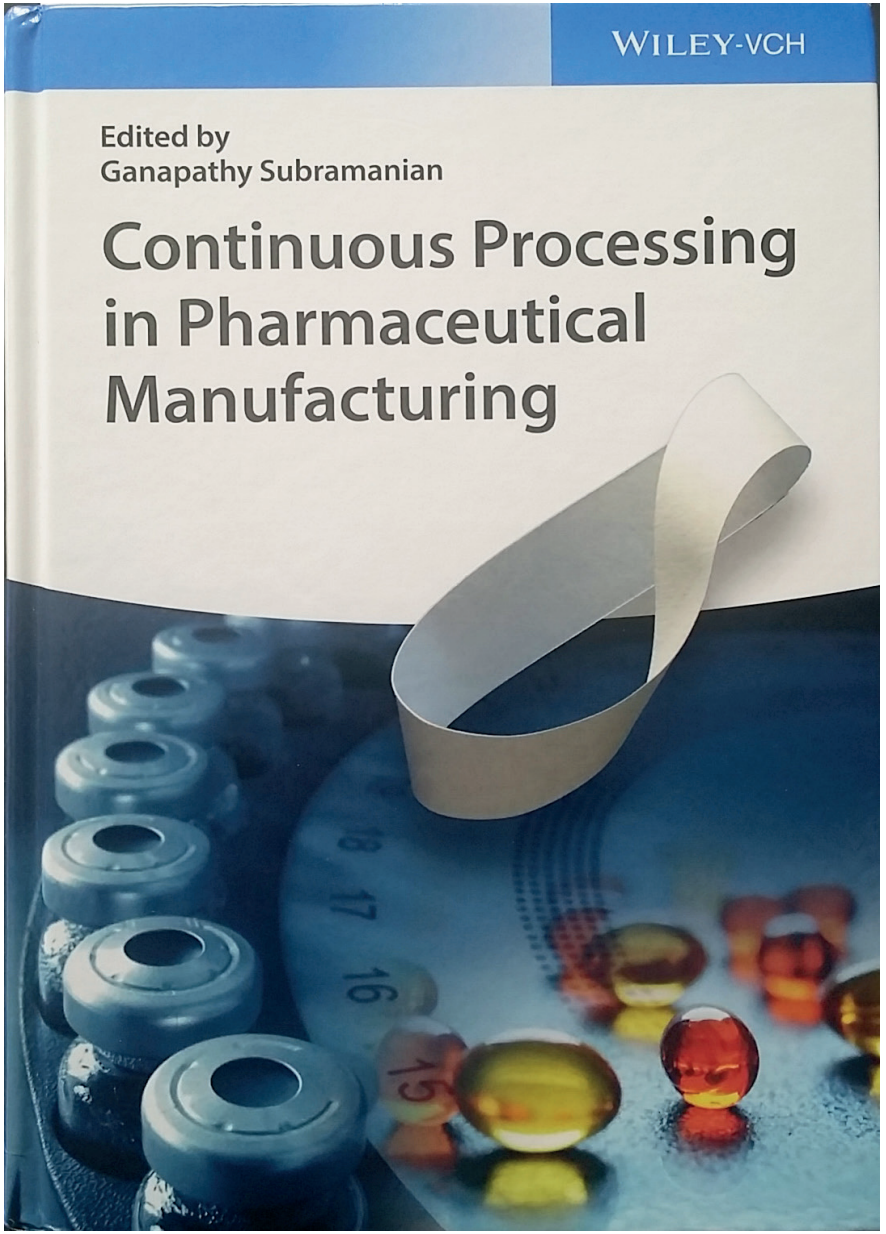
Check out www.hesub.eu

CellCore + Mnemosyne

- Mnemosyne diaphragm pump convey all liquids - (no peristaltic pumps required !)
- Diaphragm pump able to harvest or bleed cells - avoid completely Trypsin !!
- Next generation of Single-Use-Sensor´s for pH, DO, Glucose, Lactate, biomass
- 100 % single-use
- 100 % Continuous Processing



Thanks Subi



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Presentation as of June 27th 2016