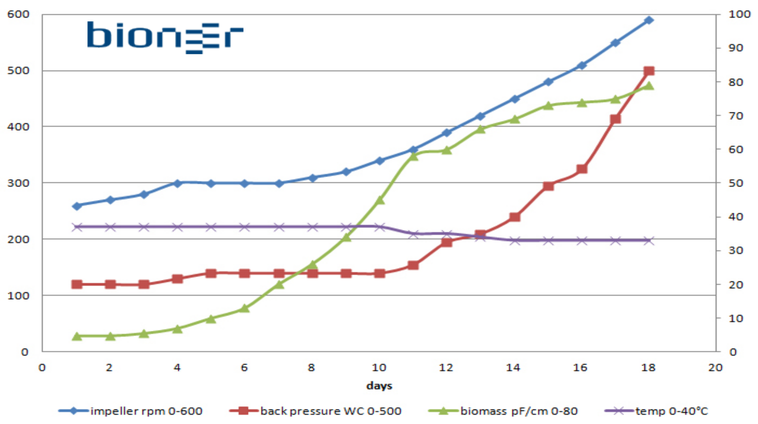
# Single-Use-Bioreactors

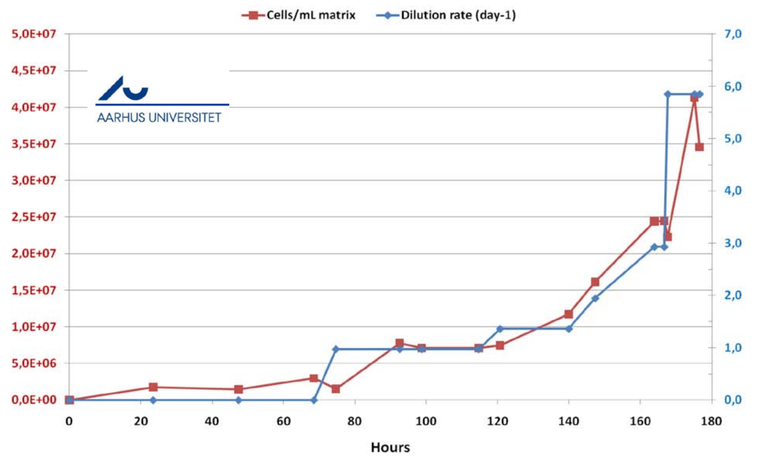
From an engineering perspective, the challenge is to eliminate the bottleneck of mass transport limitations of cell's access to oxygen and nutrients set by the requirement of the mammalian cells to increase both cell growth potentials and productivity. From an operational perspective, the challenge is to eliminate the labor-intensive and error prone manual operations that include setting up the bioreactor, multiple off-line sampling over the entire length of the cell culture, and guess-estimating the optimal time of harvest. To help the industry to solve these problems CerCell has develop the gradient-free, novel flow path, Single-Use-Bioreactor, perfusion platform available from bench-scale to pilot scale. An even number of envelopes with permeable wall contain the porous scaffolding harbouring high density suspension as well as adherent cells. The patented design mimics the packed bed bioreactor but removes the current limits on gas and mass transfer experienced with the traditional bioreactor design.

## SUB platform for high cell density

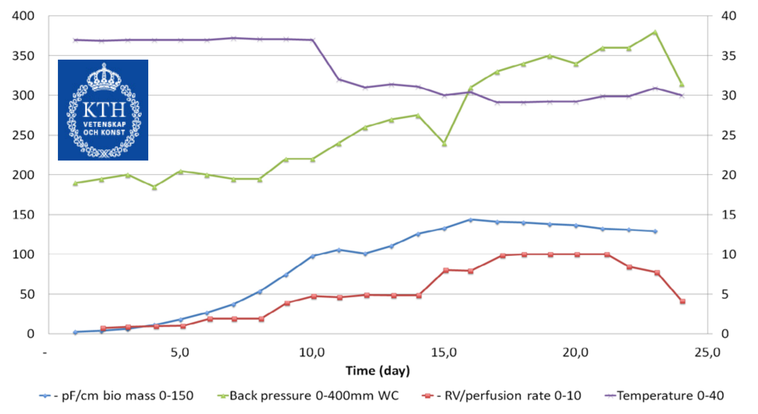



*Graph 1 - Relation between impeller rpm (in order to keep constant flux at 20 cm/min) and increase in bio mass (measured in pF/cm = 1x10E+08 cell/ml) and back pressure acros the matrix.*

At Bioneer the SUB was mounted on the DasGip M10 magnetic stirrer table at 37°C. The optical dO2, chemical pH sensors was calibrated and the SUB kept the night over with impeller revolution at 260 rpm with 30% O2/70% N2 gas mix at 3 l/h flow. The SUB was inoculated with a total of 7.38x10E+08 suspension CHO cells equivalent to 4.9x10E+06 cells/ml matrix or 5.9x10E+05 in the total reservoir volume. The re-circulation mass flow was set by 260 rpm impeller speed corresponding 1.0 l/min media re-circulation mass flow equivalent to 10 cm/min flux in order to distribute the cells inside the matrix. First recorded reading on the Fogale i465 display was 70 minutes after last inoculation and showed 4.6 pF/cm indicating cells was being trapped inside the matrix. The added volume of 333 ml was removed and the SUB was set to operate in batch mode. A sample was taken from the reservoir showing 3.9x10E+04 cells/ml. On day 11 the perfusion flow was increased to 4 l/24h, re-circulation flow kept at 2 l/min now at 360 rpm and the temperature kept at 35°C. On day 15 the temperature is continuing at 33°C and the bio mass figure at 73 pF/cm slightly increasing. Needed rpm in order to overcome the matrix / biomass backpressure of 295 mm WC and 2 l/min media re-circulation media flow was 480. A balance of temperature and glucose addition was reached.

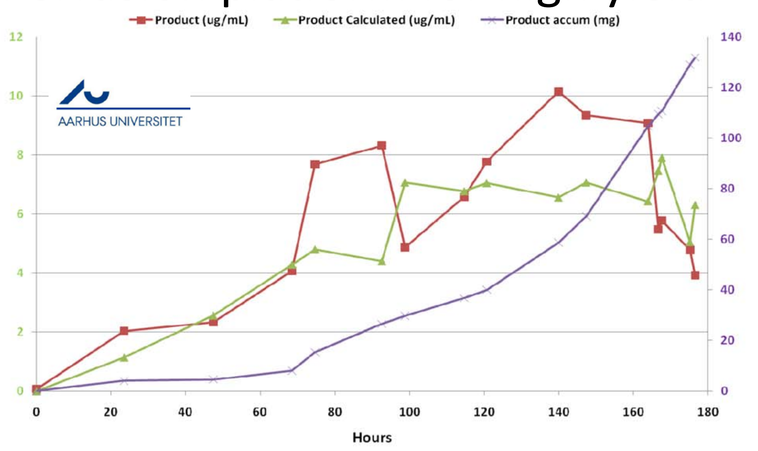



*Graph 2 - The 480 ml matrix volume CellTank at Aarhus University (january 2011) was seeded with HEK293 adherent cell line with a total of 4x10E+08 cells of viability >95%. The re-circulation flow was set for the experiment with at flow of 1,000 ml/min (4 cm/min flux). Seed density in the non-woven PET matrix was ~0.8x10E+06 cells/ml matrix volume. The cell density over the experiment increased to >4x10E+07 cells/ml matrix or in total 2x10E+10 cells. Medium perfusion exchange was started at day 3 at a rate of 0.5 reactor volume/day (with DMEM medium) increased to 6 reactor volume/day at experiment end.*

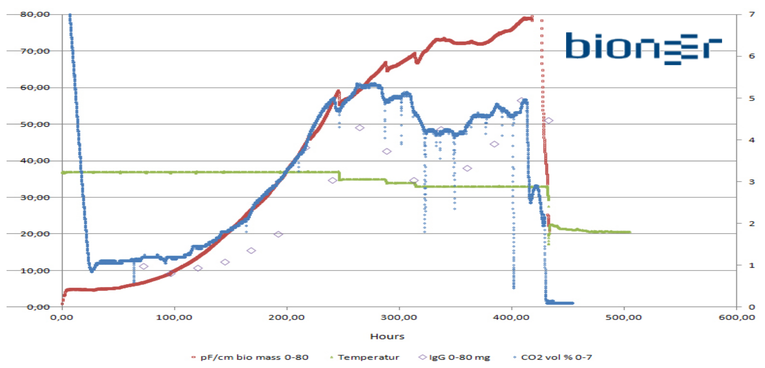



*Graph 3- At the Royal Institute of Technology, Stockholm, Sweden (december 2011) the 150 ml (RV) matrix CellTank (1 litre wv) was operated with a suspension CHO cell. 1x10E+08 cell/ml matrix was reached at 37°C day 13. Temperature then lowered and kept constant at 30°C for further 11 days at 1.3x10E+08 with perfusion rate ranging 8-10 RV/day. Expression of IgG over each of the 11 day cultivation was 37 mg/liter media. A parallel shaker flask expressed 37 mg/liter total for the 6 day cultivation. 4 RV/day is too low in order to keep lactate levels low.*

## Perfusion platform for high yield




*Graph 4 - Adherent HEK293 cell expression evaluated manually in samples with ELISA based assay of stable transfectant with proprietary gen product in mg/liter perfused media.*




*Graph 5 - On-line bio mass pF/cm, temp °C, CO2 and daily sample specific IgG productivity in tenth mg/day.*

The SUB at Bioneer express continuesly the same amount of product per day as the batch STR in parallel over 6 days. The SUB operated over several days at 70-80 pF/cm = 0.7-0.8x10E+08 cell/ml/matrix at 33°C with app 1 g/liter glucose and ~40 mMol lactate. The suspension CHO cell line was able to express IgG at temperature as low as 33°C with limited proliferation. The SUB produced 0.52 gram product over 15 days with 31 litre spent media being 11 times more than the STR batch which produced 0.045 gram antibody based on 0.6 liter media.

References:
• Bioneer A/S, Hoersholm, Denmark, senior scientist Holger K. Riemann, www.bioneer.dk
• Royal Institute of Technology (KTH), Sweden, Ph.D. Veronique Chotteau and Ye Zhang from www.biotech.kth.se
• Aarhus University, Denmark, Ph.D. Mikkel Holmen Andersen, http://mb.au.dk/en
• CMC Biologics, Soeborg, Denmark
• NovoNordisk, Maaloev, Denmark

After decades of product research and development, the cultivation of mammalian, insect and stem cell in bioreactor remains a challenge on multiple fronts. The methods of growing cells in bioreactor essentially have remained unchanged since its inception. The traditional Stirred-Tank-Reactor (STR) design suffers from mass transport limitations because of limits on agitation and sparging. And the conventional bioreactor control strategy suffers from the lack of automation features such as software capability and advanced sensing so the mode of cultivation is still overly reliant on manual labour.

## Scalable perfusion platform for suspension and adherent cell lines

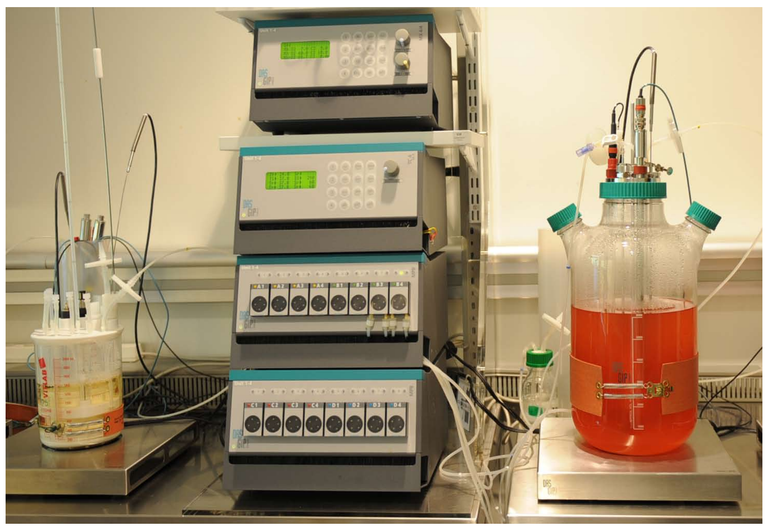
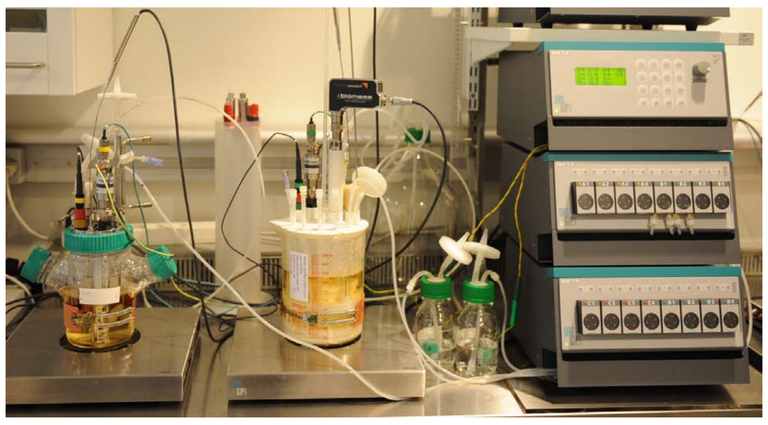


*Photo 1 - Lab testing at Bioneer in Denmark (november 2011) of the CellTank p/n 22-0150 perfusion SUB on DasGip MP8 Process-Control-System (PCS) with identical suspension CHO cell line compared against an autoclaved 8 litre glass STR. Both STR and SUB had identical 1.5x10E10 cells. The SUB with 150 ml matrix volume/1 litre reservoir volume operates for weeks in perfusion setup. The traditional STR with CHO cells in suspension and 6 litre working volume was cultivated for 6 days before termination as to extensive lactate levels.*



*Pnoto 2 - CellTank p/n 22-0150 SUB tested at Bioneer on DasGip MP8 PCS with suspension CHO producing IgG compared against a 600 ml working volume conventional glass/steel STR. The SUB reached cell density of 0.8x10+08 cells/mL for continues perfusion of 2 litre exchange/day. Graph 1 and 5 at left. Application Notes on www.cercell.com*

*Photo 3 - 480 ml matrix volume early CellReactor enclosed in an Applikon STR and controlled by a BBraun Biostat PCS at Aarhus University in Denmark. The 40 watt Applikon P100 servo motor operate easily the CellReactor in the required 250-600 rpm range. Graphs 2 and 4 seen at left illustrate the gained data. Application Notes on www.cercell.com*

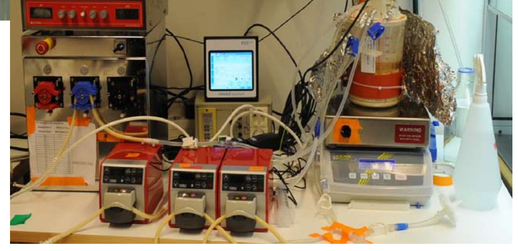


*Photo 4 - A CellTank p/n 22-0150 controlled by a wide selection of Belach PCS at Royal Institute of Technology, Stockholm in Sweden. The EVO display with the blue screen is highly visible in the picture centre for on-line measurements of the bio mass. Graph 3 at left show excellent control of proliferation for weeks of product expression.*

## Single-Use-Sensors

The highly sophisticated cell based production processes need to be monitored and controlled to guarantee product quality and to satisfy GMP requirements. With the Process-Analytical-Technology (PAT) initiative, requirements regarding process monitoring and control have changed and real-time in-line monitoring tools are now recommended.

*Sketch 1 - 3D cut through the CellTank p/n 22-0150 illustrating at left the 120 mm pH SUS and at right the re-usable Visiferm PG13.5x160 dO2 sensor fitted to the non-invasive well with the permeable membrane in the front. Slightly visible behind pH is the 120 mm SUS bio mass sensor.*

For ease of use Hamilton sensors in CerCell products Single-Use-Sensors (SUS) for dO2 and pH measurements offer the following advantages:

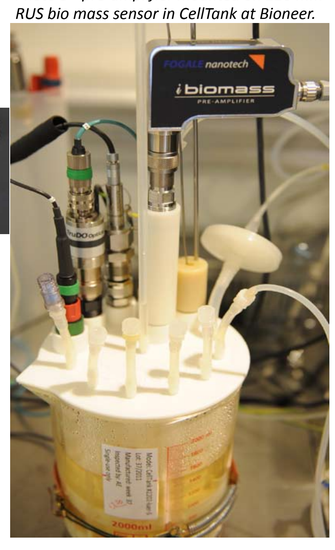
• Integrated SUS eliminate contamination risk
• Save hours of prep time and labor, as no autoclaving or cleaning is needed
• Enable SUB setup right on the bench – no biosafety cabinet / hood needed for operation
• Semi-permeable membrane at end of non-invasive well for re-usable VisiFerm classical DO probe
• Extend DO probe life, as it is never autoclaved
• Classical pH probe for extended lifetime needed for months of perfusion cultivation
• Classical pH and dO2 sensor signal fit any PCS




*Photo 5 - pre-amplifier mounted on 220 mm RUS bio mass sensor in CellTank at Bioneer.*

*Photo 6 - The well established bio mass stainless steel Re-Usable-Sensor (RUS) and below the white bio mass SUS. Both engineered in classical format PG13.5 x 120 mm*

CellTank´s integration of Fogale's single-use capacitive sensing technology allows precise on-line monitoring of the cell mass, viable cell density as well as cell physiological state. Users can also track cell cycle changes, model apoptosis, and predict protein titer all in real time, and this from inside the scaffolding matrix.



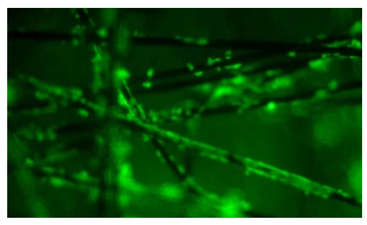

References:
• Fogale BioTech, Cambridge, MA, USA - www.fogale.com
• Hamilton Sensors, Bonaduz, Switzerland - www.hamilton.ch
• Belach, Stockholm, Sweden - www.belach.se
• Svanholm instruments, Denmark - www.svanholm.com

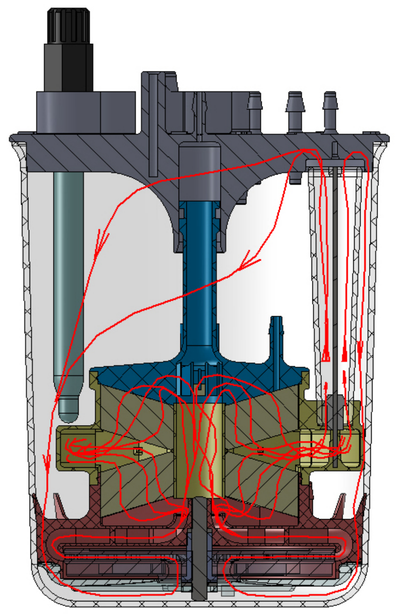
## CellTank perfusion SUB platform scalable 1:100

Reactor core design is a cylinder with stacked even number of circular envelopes arranged parallel with radial inlet and axial outlet. The core is inserted in a slightly conical beaker vessel. We call the principle core technology for CellCore.

Media pump inlet is at the very bottom. The media passes the advanced impeller driven by external magnetic forces. Media exits the pump into the reactor core centre to the triangular volumes above and under the envelopes and flows further perpendicular through the envelopes integrating the matrix. After the matrix media is collected from and in between the two envelopes and radial guided into the hollow circumference collection volume in direct correspondence with the rotameter.

### Perfusion with suspension or adherent cell lines





*Sketch 2 - The pump impeller a bottom operate in the 150-800 rpm range and re-circulate media in a full vortex rotation symmetrical around the reactor centre line in side the rigid wall vessel.*

CellTank replaces the conventional thinking of short time batch and creates the future single-use extended time perfusion platform. CellTank ranging 150 ml to 1,500 ml matrix volume and future 15 litre in just one platform is radically a satisfy all cultivation needs for research or pilot scale production, etc.

All CellTank products are engineered:
• to increase volumetric productivity with 1:10 - 1:50 on your existing PCS in your existing facilities eliminating further investments in larger PCS and lab facilities
• with integrated classical signal SUS pH probe, dO2 / non-invasive well and bio mass probe
• as high precision E-beam sterilized and disposable product ready to use right out of the bag
• to operate with a variety of turn tables or servo motor drives

## CellVessel single-use STR platform scalable 1:100

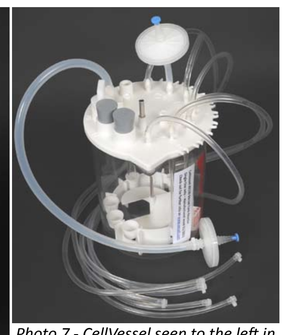
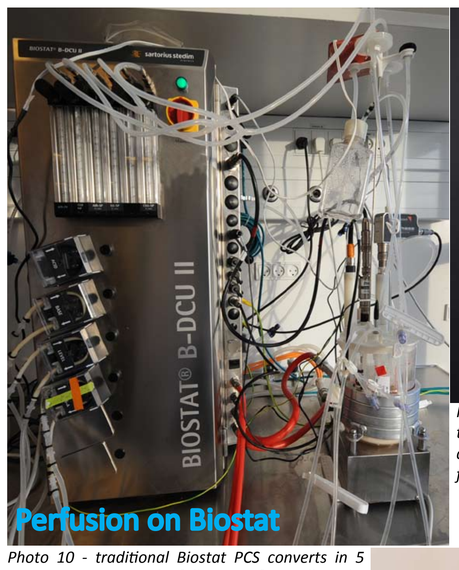


*Photo 7 - CellVessel seen to the left in 50 ml and 1,500 ml working volume all with Luer-Lok connections.*
*Photo 8 - CellVessel above with weldable silicone hoses.*

CellVessel family break the conventional thinking in re-usable STR and create the future single-use STR. The range from as small as 50 ml to 6,000 ml working volume in just one platform is radical and satisfy any need to cultivation and fermentation for research or small scale production, etc.

All CellVessel products are engineered to:
• operate 100% identical to any traditional and autoclaved glass/steel STR
• fit in between your cell lines and your existing Process-Control-System (PCS)
• operate with a variety of turn tables or servo motor drives
• use classical format and signal sensors with PG13.5 thread - RUS and SUS as you wish
• be high precision E-beam sterilized and a disposable product ready to use right out of the bag

## Single-use CellTank and CellVessel is easily adaptable to all PCS



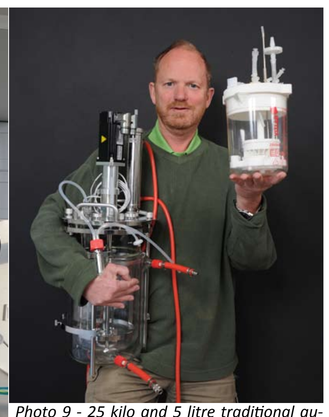



*Photo 9 - 25 kilo and 5 litre traditional autocavable, re-usable, glass/steel, batch STR compared against 750 gram single-use perfusion CellTank of equal daily expression.*

*Photo 10 - traditional Biostat PCS converts in 5 minuttes to operate the full range of CerCell single-use SUB and STR for fermentation and cultivation. No software changes is needed as the Magnetic-Stirrer-Table is driven by the Kollmorgen servo motor from the Biostat.*

*Photo 12 - light weight and high precision E-beam irradiated and ready to use.*


*Photo 11 - Dual channel bioPOD PCS from Fogale engineered for both fermentation and cultivation with single-use CellTank and CellVessel. The Magnetic-Stirrer-Table has no rotating parts and high power transfer.*

www.cercell.com for more information
Poster for ESACT-UK january 2012