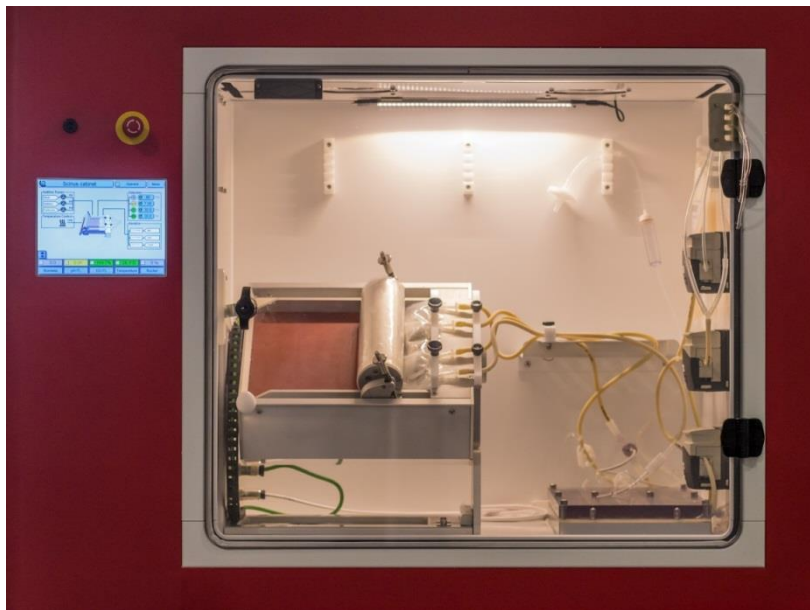




SCINUS

CELL EXPANSION



Scinus Cell Expansion system

Features

- ❑ Closed, controlled single-use bioreactor platform for GMP cell therapy production
- ❑ Designed for large-fold expansion: the only bioreactor for cell expansion from biopsy to clinically relevant cell numbers using step less volume expansion
- ❑ Unique agitation system superior to other micro carrier based solutions
- ❑ Fully customizable to optimally suit your cell type
- ❑ Real-time monitoring and control of important cell culture parameters
- ❑ Reduce cost for operator involvement, medium use and clean rooms
- ❑ Certified (UPS Class VI) single-use bioreactor bag

Introduction

Cell therapies typically require hundreds of millions of cells for one application. These cells are obtained from donors, but initial cell numbers are extremely low. Therefore, these cell numbers need to be increased dramatically before they can be administered to the patient. Standard flask-based cell culture is extremely inefficient for cell therapy production. Flasks require heavy operator involvement and expensive clean room infrastructure, while increasing risk of contamination and operator-related variability. Process automation using closed bioreactor technology can reduce costs and improve quality of cell therapy products. The Scinus Cell Expansion system is a closed, microcarrier-based bioreactor for reliable and efficient expansion of adherent cells from minimal initial cell numbers to clinically relevant amounts. The system's unique volume-expansion capabilities make it possible to cultivate minimal cell numbers (e.g. biopsies) to clinically relevant cell numbers (close to 2 billion cells) without the need for passaging.

Scinus Cell Expansion system overview

The Scinus Cell Expansion system consists of a single-use bioreactor bag within a controller cabinet.

Single-use bioreactor bag

The single-use bioreactor bag (Figure 1) is the heart of the Scinus Cell Expansion system. The bag is part of a closed perfusion loop that maintains pH and DO. It also includes containers for fresh medium, waste containers for spent medium and connections for sampling of medium and cells. Sterile welding maintains a closed environment for every operation during culture (addition of cells, microcarriers and medium, removal of excess waste, sampling, etc.). In addition, sterile welding is also used to integrate the SCINUS in the complete production cycle, including upstream and downstream processes (USP/DSP).

The bioreactor bag has integrated sensor technology that monitors the culture environment at the point of cell growth.

The bioreactor bag defines the volume available for cell culture. The volume can be limited clamping the bag (Figure 2, left), and can then gradually be increased to accommodate a growing cell population. The volume can be increased more than ten-fold to over 1L (range 80 mL – 1350 mL, Figure 2, right).

A schematic presentation of the perfusion loop is shown in Figure 3.



Figure 1 The single-use bioreactor bag of the Scinus Cell Expansion System, with incorporated sensors to the right

The single-use bag and tubing feature:

- USP Class VI plastic throughout
- Integrated sensors for pH, oxygen and biomass
- One inflow port for addition of medium
- Two outflows with filters for medium removal and harvest
- One outflow for removal of gas formations
- Tygon weldable tubing for connections to
 - Fresh medium addition
 - Waste removal
 - Sampling
 - Third party USP/DSP systems
- Pressure sensor



Figure 2 Expansion of the volume of the single-use bioreactor bag. Minimal volume (left) and maximum volume (right).

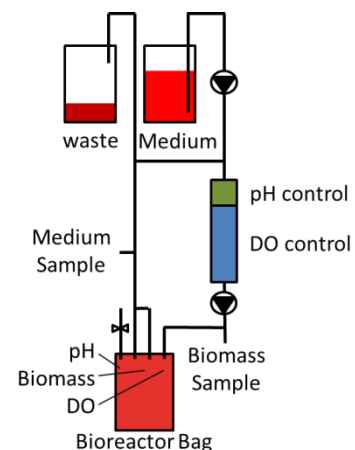


Figure 3 Schematic presentation of the perfusion loop of the Scinus's single-use bioreactor bag

Controller cabinet

Culture inside the bioreactor bag is done in a hardware enclosure (Figure 4). This hardware enclosure houses a unique expansion platform, integrated controller software, an oxygenation system, and all other required hardware such as pumps and heaters.

- Full touch screen interface for parameter setting with different login levels (operator, engineer, service, etc.)
- A unique agitation (rocker) platform that also houses the integrated transmitters for sensors
- Control loops for pH, oxygen and temperature
- A patented oxygenation system for accurate gas control
- Integration with third-party software through Ethernet
- Modest footprint: 108 x 82 x 47 cm



Figure 4 The Scinus Cell Expansion controller cabinet

Expansion platform

A homogenous cell culture environment is maintained by a unique platform (Figure 5). The bioreactor bag is positioned on a platform that can rock, thereby mixing the content of the bag and maintaining a homogeneously distributed cell suspension. The settings of agitation can be optimized for every individual cell culture application, depending for example on cell type, growth speed or sensitivity to shear stress. The expansion platform houses the clamp that is used to define the available culture volume inside the bioreactor bag. The clamp can be automatically repositioned to increase or decrease the available volume.



Figure 5 The Scinus's expansion platform

Expansion platform parameters

Parameter	Range
Volume range	80 mL – 1350 mL
Rocking angle	0 – 180 °
Rocking speed	1 – 500 °/s
Acceleration	10 – 1000 °/s ²
Deceleration	10 – 1000 °/s ²
Horizontal pause	0 – 7200 s
Horizontal cycle count*	0 – 5000
Vertical pause	0 – 7200 s

* Number of rocking cycles between two horizontal pauses

Environmental control

The Scinus maintains an optimal culture environment with strict control of temperature, oxygen and pH levels. Oxygen and pH levels are measured by the sensors that are integrated in the single-use bioreactor bag. Transmitters are built into the rocking expansion platform and generated data is transmitted to the controller software of the cabinet. The controller software subsequently maintains the pre-defined culture environment by controlling the addition of gases. A patented oxygenation system ensures that set points are maintained within minimal bandwidths. The system's unique rocking approach ensures minimal levels of shear.

- Accurate control of pH, oxygen and temperature
- Homogenized culture through rocking with minimized levels of shear
- Temperature control
- Mass-flow controllers for gas flow control

Parameter	Requirements
DO control	N ₂ and compressed air or O ₂
pH control	CO ₂ + optional alkali pump
DO range	0 – 100%
pH range	5.5 – 8.5
Temperature range	Ambient to +45 °C

Recipe management and data acquisition

The Scinus Cell Expansion system can be linked with third-party software for recipe management and data acquisition through an Ethernet connection. The system is fully OPC compatible and 21CFR part 11 compliance is met for complete audit trails and batch records.

Operational performance

Large-fold expansion

The Scinus Cell Expansion system leverages the unique phenomenon of bead-to-bead transfer observed in microcarrier culture. This phenomenon, coupled with the system's unique volume expansion, allows users to culture adherent cells from limited amounts to high cell numbers without the need for enzymatic digestion and reseeding. The system has been shown to support the culture of 25 mL bone marrow to over close to 2 billion MSCs in a single culture (range 1.37-1.73 billion MSCs, Figure 6).

Representative sampling

A major limitation of many automated cell culture systems is the lack of sampling capabilities. Cells that adhere to a fixed surface usually cannot be partially harvested for quality control. The Scinus Cell Expansion system however enables representative sampling of both cells and medium at any point during culture. These samples can then be used for quality control assays such as phenotyping and potency assays.

Real-time biomass monitoring

Obtaining sufficient cells for any application is crucial. Traditionally the time-point of harvest is dictated by subjective assessment of confluence or, in the case of most bioreactors, through measurement of concentrations of glucose and/or lactate. The Scinus Cell Expansion system houses a capacitance-based biomass sensor that provides real-time feedback on the cell concentration (Figure 7). Offline measurements or subjective assessments of confluence are no longer necessary.

Controlled, homogeneous environment

The system's unique agitation approach maintains a homogeneous environment through constant mixing of the bioreactor bag. The gentle mode of agitation provides a fully homogeneous culture, but minimizes the shear that is usually associated with microcarrier cultures (Figure 8). Incorporation of sensors pH, oxygen and temperature at the site of cell culture ensures cells experience an optimal environment.

Flexibility

The Scinus Cell Expansion system is extremely flexible. Users can optimize the culture environment (medium, pH, oxygen, agitation etc.) to specific culture needs. In addition, different microcarriers can be used depending on the

specific substrate requirements of the cells. Therefore the Scinus can be optimized for the culture of various cell types.

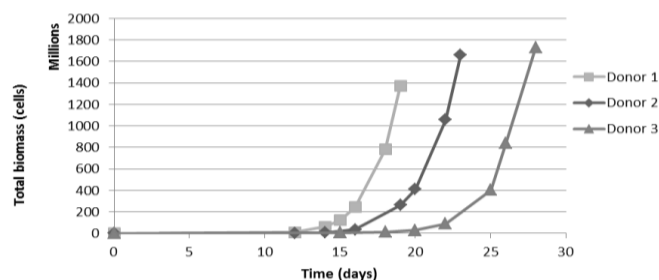


Figure 6 Large-fold expansion of MSCs in the Scinus Cell Expansion system. 25 mL of bone marrow was expanded to up to 1 billion cells (range 1.37-1.73 billion) without passaging.

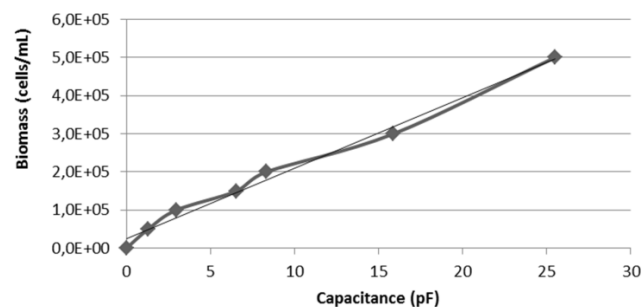


Figure 7 Capacitance, as measured by the integrated biomass sensor, as predictor for cell concentration inside the bioreactor bag. Coefficient of determination, $R^2=0.989$

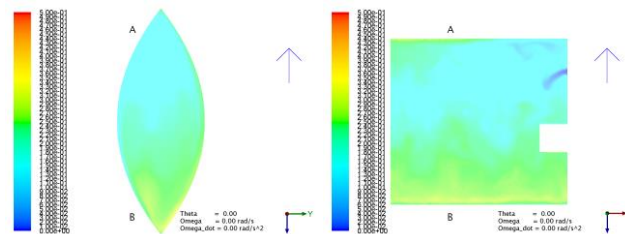


Figure 8 Computational Flow Dynamics of the single-use bioreactor bag during operation reveal a highly homogenous culture environment



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