CellScrew®

Scalable and
sustainable
adherent cell expansion system

Advantages

- **Large growth surface area** and optimal cultivation conditions, facilitating scale-up
- Easy-to-handle system requiring little additional equipment, **reducing labor costs and manufacturing space**

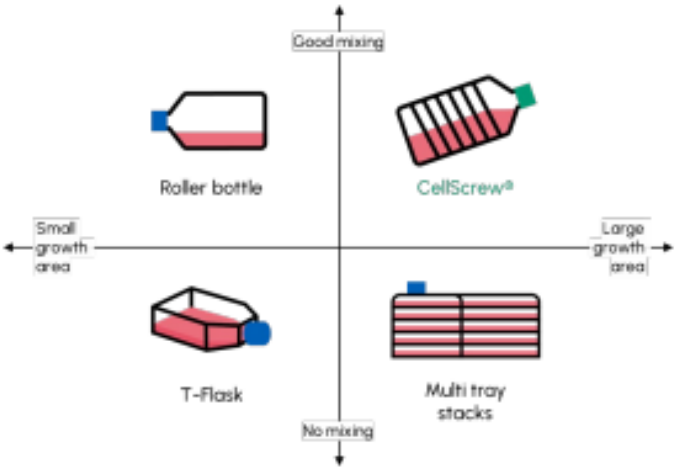
- Made from plant-based PLA, a sustainable alternative to petroleum-based plastic labware
- The CellScrew® is 3D printed, saving raw material during production

This document contains:

Introducing the CellScrew®

Scalability and Cost Efficiency

The CellScrew®'s innovative structure creates an easy-to-handle, **high surface area to volume ratio** adherent cell cultivation system. The system requires little to no additional equipment or protocol optimization when scaling up compared to existing systems such as bioreactors. Using the CellScrew® also requires **less media and harvesting enzyme**, increasing its cost efficiency (see HEK-293 Cultivation Application Note).



The Environmental Benefits

The CellScrew® is made of 3D printed plant-based biopolymer Polylactic Acid (PLA) produced entirely from renewable crops. Production using the additive manufacturing of this material allows for increased growth area using less raw material. This **reduces carbon emissions by up to 90%** compared to manufacturing of petroleum-based plastics.

Dimensions

CS6K

Cell culture area: 6,000 cm^2

Media volume: 250 – 800 mL

CS10K

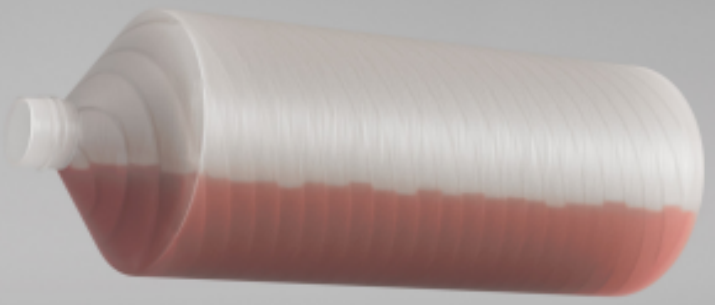
Cell culture area: 10,000 cm^2

Media volume: 450 – 1,300 mL

The Ideal Cultivation Environment

The Innovative Structure

The CellScrew®'s novel internal structures comprising of multiple Archimedean Screws, concentric cylinders and a central tube creates a **low-shear stress, continuously mixed, highly oxygenated environment**. This creates conditions for optimal adherent cell attachment and growth (see Process Engineering Characterisation Application Note).



Can all adherent cells be cultivated in the CellScrew®?

Yes, the CellScrew® has been tested with multiple different cell lines (see table below). Ask for our **free material sample** 12-well plates with PLA inserts to test the attachment and growth of your cell line.

Cell Line	Attachment*	Growth*
HEK-293	Improved	Improved
HeLa	Improved	Improved
Murine Colon Carcinoma	Improved	Improved
Murine Melanoma	Improved	Improved
VERO	Similar	Similar
hiPSC PLA coated with Matrigel	Similar	Similar
Mycoplasma pneumoniae	Similar	Similar
Mammalian Kidney Cells	N/A	Similar
Mosquito Cell Line	N/A	Similar
Human Lung Carcinoma Cells	N/A	Similar

* = compared to polystyrene 12-well plate

Frequently Asked Questions (FAQs)

Is the CellScrew® GMP-certified?

The CellScrew® is currently research grade, but we aim to achieve GMP compliance by the end of Q1 2024 and are in the process of constructing a GMP-compliant facility to support this goal.

Can cells be visualized under the microscope in the CellScrew®?

The CellScrew® cannot be microscoped, similar to most other expansion systems that exceed a growth surface of ~300 cm^2. We recommend using our free 12-well sample plates with inserts to characterize your cell growth and medium on the TC-treated surface.

Is PLA safe for my cells' cultivation?

PLA is a bioplastic that has received FDA approval as non-toxic to biological fluids. It is considered non-hazardous and stable under standard conditions. Moreover, PLA has already been widely used in medical applications, including drug delivery, tissue engineering, implants, and surgical sutures.

Do I need to buy additional equipment to use the CellScrew®?

The CellScrew® is handled just as a regular roller bottle, where it is placed in an incubator on a roller device. However, it is crucial to position the roller bottle at a a 5–10-degree angle on the roller to ensure the filter cap remains dry and the system operates effectively.

You can see the handling here: GEB I Handling

Is the CellScrew® sterile?

Yes, the CellScrew® comes fully sterilized, as it is vacuum sealed, and gamma irradiated. We strongly advise against autoclaving the CellScrew® as it can cause damage to the product's structure and integrity.

Any questions left unanswered?
Reach out!

info@hp-ne.com
+1.401.349.4477
www.hp-ne.com

Cultivation and expansion of HEK-293 cells in the CellScrew® 6K

Application of PLA as a growth surface for adherent cells

Industry Segment: Biotechnology R&D
Application Field: Cultivation of HEK-293 cells
GEB Product(s): CellScrew®

Sustainable plant-based growth surface shows cell attachment and growth comparable to standard polystyrene surfaces

Presenting a new cultivation system like the CellScrew®, made from a polymer not commonly used in cell culture applications, raises the question of system performance in comparison to standard cell culture surfaces. To address these questions HEK293 cells (DSMZ-German Collection of Microorganisms and Cell Cultures GmbH: ACC 305) were cultivated on PLA material-sample inserts for a 12-well plate and the CellScrew® 6K. The HEK293 cell line was established from human embryonal kidney cells and is widely used in academia and industry e.g., for viral vectors and transfection. The maximum cell density, the attachment time, and the increase of cell density over time of HEK293 cells were investigated in RPMI 1640 medium (with 25mM HEPES) + 10 % FBS + 1 % Penicillin-Streptomycin. This dataset gives a first overview about the suitability for the TC-treated PLA Surface and the CellScrew® cultivation system to replace polystyrene systems in research laboratories, process development and biopharmaceutical production.

Advantages of the CellScrew®

- Attachment of HEK-293 in the CellScrew® was successful
- Expansion of HEK-293 in the CellScrew® was successful and exceeded DSMZ data
- No protocol modification necessary for trypsinization
- Harvesting enzyme concentration was reduced by 66 %

All data was collected and analyzed in-house.

Authors: Björn Boshof, Joel Eichmann, Lukas Käßer

Experiment setup

The R&D Department of Green Elephant Biotech GmbH planned and executed experiments in-house. The experiments were performed in the CellScrew® 6K, PLA 12-well material sample inserts and standard PS 12-Wells with RPMI 1640 (with 25mM HEPES) + 10 % FBS + 1 % Penicillin-Streptomycin. Cultivation conditions were 37 °C and 5 % CO2 atmosphere. For the CellScrew® these conditions and the 0.5 rpm rotational speed were controlled in a drive-unit for bottles with incubating hood (INCUDRIVE D-1 CO2, schuett-biotec GmbH). The 12-well plate and the PLA material-sample inserts were monitored, and three wells of each type were harvested in a 24 h interval. To harvest the cells DPBS, Trypsin/EDTA (1x) and serum-containing medium was used. Additionally, the medium was exchanged every 24 h for all CellScrew® 6K starting 48 h after seeding. Harvest of the CellScrew® 6K to obtain growth kinetic data started 48 h after seeding and was executed with a reduced trypsin concentration (0.33x).

Results

HEK-293 were cultivated in 12-well material sample inserts and wells of a polystyrene 12-well plate. Seeding density was 66,000 cells* cm^{-2}, the cells were cultivated over 4 days to compare the growth on both, the polystyrene, and the PLA surface. Figure 1 shows the growth kinetics for both experiments. Maximum cell densities of ~ 440,000 cells*cm^{-2} and ~ 530,000 cells*cm^{-2} were achieved which doubles the data from DMSZ that shows ~ 250,000 cells*cm^{-2} after ~ 3 days in culture. The maximum growth rate in both plates was comparable.

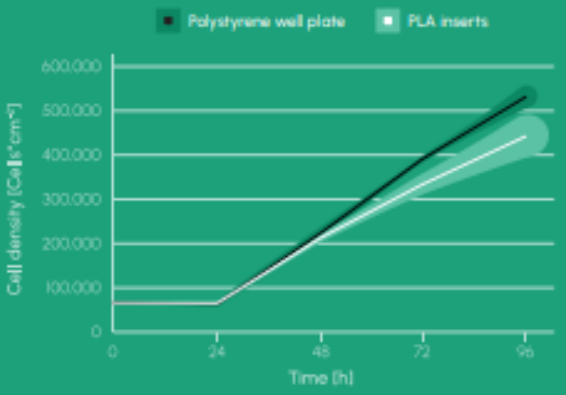


Figure 1: Growth kinetic of HEK-293 in 12-Well PLA inserts and a polystyrene 12-well plate in RPMI (with 25mM HEPES) + 10 % FCS + 1% Penicillin-Streptomycin with a seeding density of 66,000 cells*cm^{-2}.

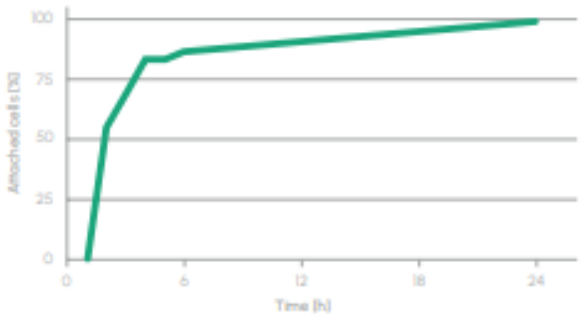


Figure 2: Attachment kinetic of HEK293 in CS6K at 0.5 rpm.

HEK293 attachment to the surface of the CellScrew® 6K was observed at 0.5 rpm rotational speed. The cell concentration in the supernatant was counted in technical duplicates. The seeding concentration was 800,000 cells*mL^{-1}. Figure 2 depicts the attachment of ~ 87 % after 6 h and almost 100 % attachment after 24 h. Since it takes ~ 24 hours for the cells to attach, a corresponding attachment phase is to be expected when cultivating the cells in the CellScrew®.

All data was collected and analyzed in-house.

Authors: Björn Boshof, Joel Eichmann, Lukas Käßer

Cultivation and expansion of HEK-293 cells

Products: CellScrew® 6K

Depicted in Figure 3, HEK-293 were cultivated in CellScrew® 6K at 0.5 rpm rotational speed with a seeding density of 66,000 cells*cm^{-2} over 7 days. Maximum cell densities were ~ 325,000 cells*cm^{-2} after 6 days and ~ 370,000 after 7 days. Because attachment of the cells in the continuously mixed system takes place in the first 24 hours, the lag-phase is prolonged by the attachment phase in comparison with the kinetic obtained from the 12-well plate experiments.

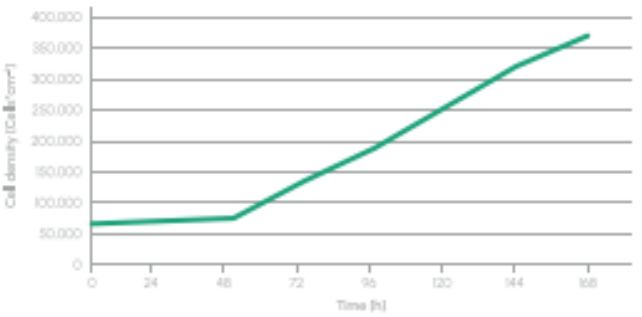


Figure 3: Growth kinetic of HEK-293 in CS6K in RPMI (with 25mM HEPES) + 10% FCS with a seeding density of 66,000 cells*cm^{-2}.

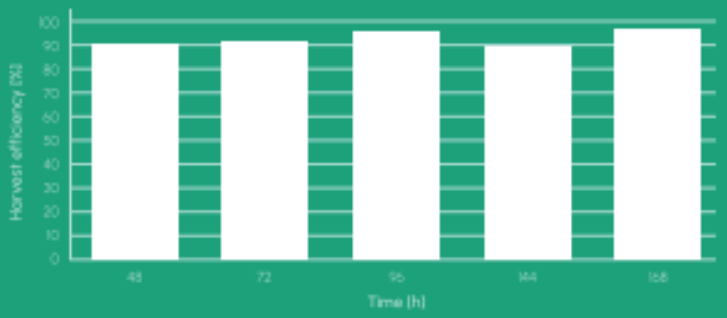


Figure 4: Harvest efficacy of CS6K HEK293 harvest using trypsin (1:3 in DPBS) with an additional washing step with DPBS after harvesting.

To determine the efficacy of the CellScrew® harvest protocol an additional washing step with DPBS was done after removing the cell suspension of the harvest. The cells in the washing step suspension were counted and the efficacy was calculated and is displayed in Figure 4.

The data presented shows that HEK-293 are sufficiently supplied with oxygen and substrates during the expansion in the CellScrew®. Cell densities exceeded the predicted number of 250,000 cells*cm^{-2} without further optimization and can potentially be improved by more sophisticated medium exchange intervals or a feeding strategy. Without adapting the standard harvest protocol, harvest efficiencies were around 90 % and above, even with a reduction of ~ 66 % in trypsin concentration.

Special thanks

We like to thank schuett-biotec GmbH for the provision of the INCUDRIVE D-I CO2 Roller Bottle Incubator with integrated drive-unit for bottles and incubating hood (bench-top) equipped with CO2-function, used in this study. The device is to be equipped with up to 4 flexible roller inserts to roll i.e., 4 (CellScrew® 10K) or 8 (CellScrew® 6K) cultivation flasks per roller insert, in total 16 or 32 CellScrew® cultivation flasks per incubator.

Dr. Lukas Käßer
tech-service@greenebt.com
Green Elephant Biotech GmbH

Kerkrader Straße 9
35394 Gießen
Germany

All data was collected and analyzed in-house.

Authors: Björn Boshof, Joel Eichmann, Lukas Käßer

Process Engineering Characterization of the CellScrew® 6K and the CellScrew® 10K

Mixing Time and Oxygen Transfer Coefficient

Industry Segment: Biotechnology R&D
Application Field: General process engineering characterization
GEB Product(s): CellScrew®

Well defined processes need prior system characterization

The Quality by Design (QbD) initiative demands the determination of critical quality attributes (CQAs) and the tight process control of the corresponding critical process parameters (CPPs). Depending on the process, the cell line or the product, shear forces, a fast homogeneous distribution of substances or the oxygen transfer can be CPPs. To be able to identify the CPPs in a process, a good characterization of the cultivation system is mandatory.
For a cultivation system like the CellScrew®, the most important process engineering parameters are the mixing time and the oxygen transfer coefficient.

Whenever working with agitated systems, a homogeneous liquid phase is assumed for calculations based on substrate concentrations, pH, oxygen saturation etc. Changes in the liquid phase occur whenever a substance or volume is taken or added to the liquid phase. To justify the assumption of homogeneity from the perspective of the cultured cells, the mixing time must be considerably lower than the doubling time of the cultivated organism.
In many cases, oxygen supply is a critical process parameter in aerobic cell culture processes, emphasizing the importance of oxygen transfer into the culture medium. The oxygen transfer coefficient provides information about the transport of oxygen from the gaseous phase into the liquid phase and the surface area per volume of liquid and is a function of agitation.

The Research and Development department of Green Elephant Biotech GmbH is constantly further investigating properties and applications of the CellScrew®, sharing information about our findings with the scientific community.

Advantages of the CellScrew®

- The CellScrew® offers **short mixing times** over the whole recommended rpm range
- The CellScrew® is **comparable with regular multiple-use benchtop bioreactor systems** regarding the k_La-value
- The k_La-value of the CellScrew® **increases** and the **mixing time decreases with increasing rotation speed**

All data was collected and analyzed in-house.

Authors: Björn Boshof, Joel Eichmann, Lukas Käßer

Comparison of mixing time and oxygen transfer coefficient (k_La-value) at different rotational speeds (rpm)

The R&D Department of Green Elephant Biotech GmbH planned and executed experiments in-house. The experiments were performed in the CellScrew® 6K and the CellScrew® 10K with water at room temperature (ca. 25 °C). Mixing time was determined optical in blind triplicates by adding food coloring into the liquid phase and measuring the time until an even distribution was observed. The k_La-value was determined by static gassing-out. After removal of oxygen in the liquid phase inside the CellScrew® with nitrogen gassing-out, the gaseous phase (nitrogen) inside the CellScrew® was replaced by regular air. Dissolved oxygen (DO) was then monitored in-line (VisiFerm sensor, Hamilton) in the aqueous phase. DO data was used to calculate the k_La-value.

Results

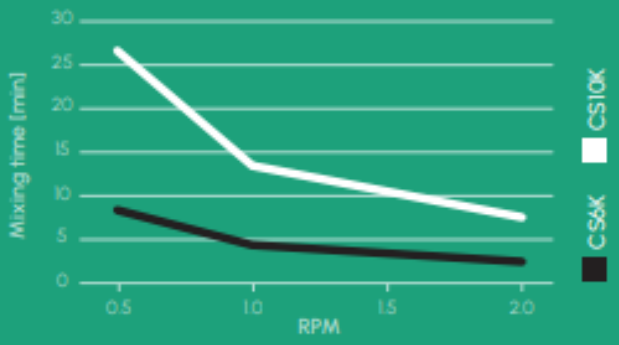


Figure 1 : Mixing time of the CellScrew® 10K and the CellScrew® 6K in the recommended range of rotational speed of 0.5-2 rpm.

The mixing time was determined in the CellScrew® 6K and the CellScrew® 10K for 0.5, 1 and 2 rpm rotational speed. Figure 1 depicts mixing times lower than 9 minutes for the CellScrew® 6K and lower than 27 minutes for the CellScrew® 10K when rotated in the recommended rotation speed range. With increasing rotational speed, the mixing time is decreasing. For adherent cell culture, these mixing times are considerably low. This justifies the assumption of homogeneity for the field of application aimed for. Furthermore, the mixing properties of the CellScrew® enable an even distribution of cells inside the CellScrew® when seeding but also a gentle, quick, and even distribution of e.g., Trypsin when harvesting the cells.

The oxygen transfer coefficients, or k_La-values, were also determined in the recommended rotation speed range at 0.5, 1 and 2 rpm. For the CellScrew® 6K a maximum k_La-value of 10.8 ± 0.7 h^{-1} was observed, while the maximum k_La-value was 8 ± 0.4 h^{-1} for the CellScrew® 10K, respectively. These values are comparable to those of benchtop stirred tank bioreactors with bubble gassing and moderate agitation. Just like expected the k_La-value increases with increasing rotational speed. The data presented shows that adherent growing cells can sufficiently be supplied with oxygen during the expansion in the CellScrew®. Especially for oxygen demanding cell lines or oxygen intensive culture processes the CellScrew® offers great potential without the need of additional aeration and increased shear stress.

We like to thank Hamilton Germany GmbH for the test equipment used in this study.

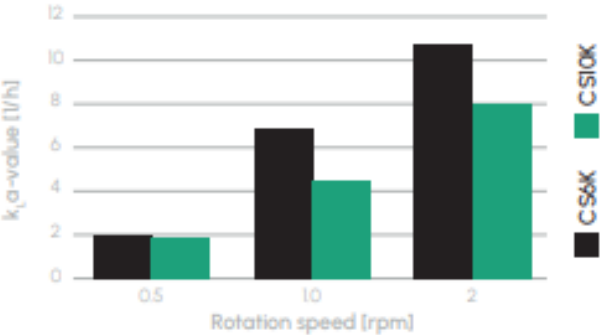


Figure 2 : Oxygen transfer coefficient (k_La-value) for the CellScrew® 10K and the CellScrew® 6K in the recommended range of rotational speed of 0.5-2 rpm.

Dr. Lukas Käßer
tech-service@greenebt.com
Green Elephant Biotech GmbH
Kerkrader Straße 9
35394 Gießen
Germany

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