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 % without loss in harvest efficiency

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**

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-I 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} and 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.

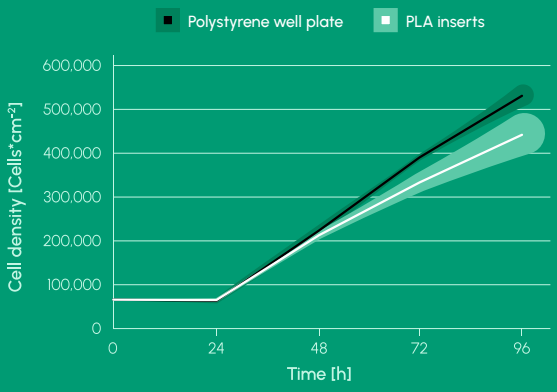

Figure 1: Growth kinetic of HEK-293 in 12-Well PLA inserts and a polystyrene 12-well plate in RPMI (with 25mM HEPES) + 10 % FCS + 1% Penicillin-Streptomycin with a seeding density of 66,000 cells*cm^{-2}.

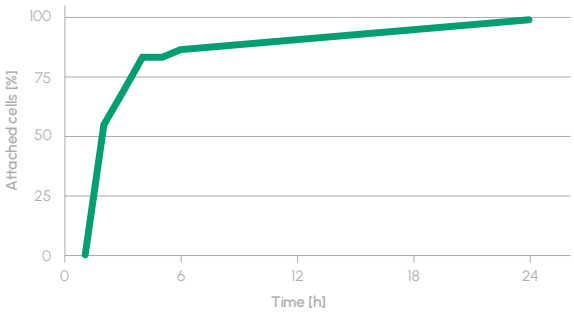

Figure 2: Attachment kinetic of HEK293 in CS6K at 0.5 rpm.

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 h 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 h, the lag-phase is prolonged by the attachment phase in comparison with the kinetic obtained from the 12-well plate experiments.

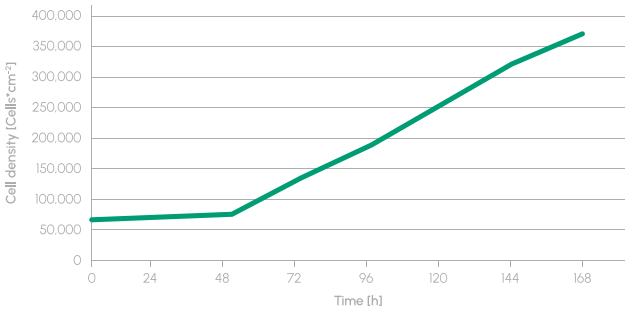

Figure 3: Growth kinetic of HEK-293 in CS6K in RPMI (with 25mM HEPES) + 10% FCS with a seeding density of 66,000 cells*cm^{-2}.

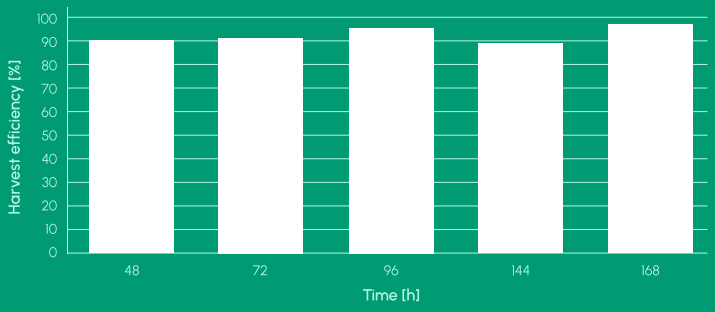

Figure 4: Harvest efficacy of CS6K HEK293 harvest using trypsin (1:3 in DPBS) with an additional washing step with DPBS after harvesting.

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.

Green Elephant BIOTECH

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