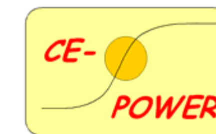
High-level expression of recombinant protein in CHO cells using the disposable CELLine *adhere* bioreactor flask



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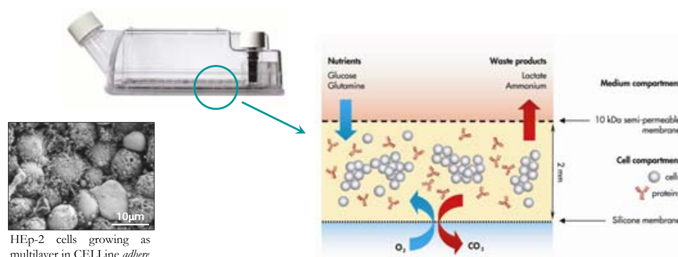
Abstract

Conventional techniques for the culture of anchorage-dependent cells allow growing cells to a maximal density of 10^6 cells per ml and expression of recombinant proteins on a milligram-scale can only be obtained either by using large numbers of standard cell culture disposables, which is a time and labour intensive process, or by working with a small scale bioreactor, which requires the handling of rather demanding equipment. To overcome the limitations of low-density cell cultivation, INTEGRA Biosciences has developed the disposable CELLine *adhere* bioreactor flask, which is based on compartmentalisation technology and allows cultivating adherent cells in a quasi-perfusion mode to densities of more than 10^7 cells per ml.

Here we present the two-compartment CELLine flask, describe a basic protocol for culturing anchorage-dependent CHO cells in CELLine *adhere* and show that expression of a secreted recombinant protein in CELLine *adhere* resulted in a nine-fold increase in productivity when compared to conventional T-flask cultures. These results and data obtained with other cell types indicate that the CELLine bioreactor is a valuable laboratory tool for expression of high levels of recombinant protein using adherent cell lines.

Two-compartment technology



HEp-2 cells growing as multilayer in CELLine *adhere*

The core of the CELLine system is the cell compartment, a bag formed by two different membranes and incorporated into the bottom of the flask. The upper membrane separates the cell compartment from the medium compartment and is made of a cellulose acetate dialysis membrane. Owing to the semi-permeable properties of the membrane, small nutrient molecules diffuse freely to the cells, while toxic catabolites are continuously removed from the cells by diffusion in the opposite direction. The lower membrane of the cell compartment is made of gas-permeable silicone that guarantees efficient oxygen and CO_2 transfer. Because the cell compartment is only 2-3 mm thin and the cells reside directly on the silicone membrane, efficient oxygenation is achieved by diffusion, without the need of mixing the culture and thereby subjecting the cells to shear stress. Furthermore, the individual accessibility of the compartments allows to supply cells with fresh medium without mechanically interfering with the culture. All together, the CELLine technology simulates quasi *in vivo* conditions resulting in high cell densities and in the production of highly concentrated recombinant protein within the cell compartment. Furthermore, for cultivation of adherent cells, the cell compartment of the CELLine *adhere* bioreactor is fitted with a cloth-like PET matrix where anchorage-dependent cells attach and grow as a multilayered cell mass (as shown above in a EM micrograph of HEp-2 cells [with courtesy of W. Pfaller, University of Innsbruck]).

CHO cell culture in CELLine *adhere*

Three CELLine *adhere* 1000 flasks were inoculated each with 1.5 x 10^8 CHO cells suspended in 25 ml high-glucose DMEM supplemented with 10% FCS. The medium compartment was filled with 400 ml high-glucose DMEM with 2% FCS (the medium change schedule is shown in Fig. 1). For control purposes, the cells were also cultivated in T-Flasks (75 cm^2) inoculated with 4 x 10^3 cells/cm^2 using 20 ml of DMEM with 10% FCS and sub-cultured by splitting 1:10 every 4 days. Because it is not possible to microscopically observe the cells in a CELLine *adhere*, growth was monitored by measuring glucose consumption and lactate accumulation. Additionally, the number and viability of the non-adherent cells floating in the cell compartment were determined.

Three days after inoculation, an average of 1.2 x 10^6 cells/ml with a viability of 47 ± 13 % were floating freely in the cell compartment. At day six the number of cells in suspension dropped to 5.2 x 10^5 (viability 37 ± 6 %) and at day 10 less than 10^5 detached cells were found (Fig. 1). The high proportion of detached and dying cells at culture start (20 % of the inoculum) is explained by both a failure of some cells to attach to the PET matrix and by lack of nutrients due to an excessive medium depletion (as indicated in Fig. 2 by the glucose content of less than 0.5 g/L at day 3). From day 10 onwards the number of detached cells remained low (< 10^5) and glucose consumption remained constant (after 4 days of growth the glucose content was constantly around 1.5 g/L).

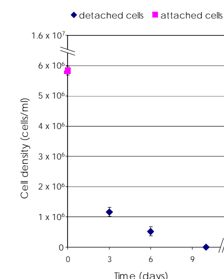


Figure 1:
Change in the density of adherent and detached cells.

To determine the density of viable adherent cells, at the end of the culture period, four rounds of trypsinisation were performed to break up the multilayered cell mass and detach all cells from the PET matrix. A total of 4 x10^8 cells or 1.6 x10^7 cell/ml (viability 97%) were recovered from the cell compartment, a density 10 times higher than compared to the T-Flask control culture.

From day 22 onward the production of recombinant protein was measured and the medium was changed at weekly intervals. During the first production week glucose levels were measured daily and, additionally, lactate accumulation was determined. As shown in Fig. 2, the level of glucose does not decrease linearly, i.e. consumption of glucose is higher in the first days after the medium change and is lower later in the week. This likely reflects a lower metabolic rate of the cells starting from day 3-4 when nutrients in the medium become depleted and the concentration of waste products such as lactate increases (lactate levels higher than 2.5 g/L are generally cell growth inhibiting).

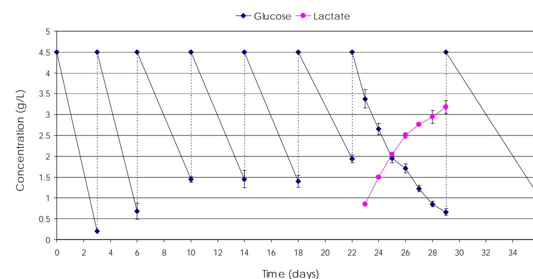


Figure 2:
Time-dependent change in glucose and lactate levels. Dotted lines symbolise a medium change (on day 3 and day 6 the medium was not changed in the cell compartment).

Production of recombinant protein

The employed CHO cells express a secreted protein X used as a vaccine, but for reasons of confidentiality the exact identity of the recombinant protein can not be revealed. To determine the productivity achieved in CELLine *adhere*, the concentration of protein X was determined by ELISA at different time points during cultivation.

Four hours after changing the medium at day 22 the product concentration was low as expected (0.04 µg/ml); when the supernatant (25 ml) was harvested after one week at day 29, the concentration increased to 65.5 ± 3.6 µg/ml, resulting in a total amount of 1.64 mg recombinant protein and a average productivity of 9.4 µg/ml*day. After one more week of culture, no significant change in productivity could be measured (the product concentration at day 36 was 57.5 ± 12.8 µg/ml). Therefore, productivity in CELLine *adhere* is approximately 9 times higher than in a non-compartmentalised system such as T-Flasks, where productivity was 1.06 µg/ml*day (Fig. 3).

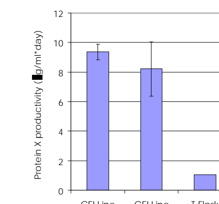


Figure 3:
Productivity of the CHO cells in CELLine and in conventional T-Flask.

Once established, a culture in CELLine can be maintained for an extended time period (2 month and more). In the case of the CHO cells used here, 6 harvests of 25 ml supernatant allow to produce approximately 10 mg of recombinant protein X, an amount typically used in research or in small-scale manufacturing. In the control experiment using T-Flasks, culture time was limited to 4 days (the time the cells formed a confluent monolayer and became quiescent) so that the maximal product concentration attained was about 4.4 mg/ml. Hence to obtain 10 mg of product with a non-compartmentalised system, more than 2 liters of supernatant need to be collected, a quantity involving a significant increase of serum consumption (compared to CELLine) and the handling of several large T-Flasks, roller bottles or a multilayer culture vessel (see Table 1).

Table 1: Comparison of compartmentalised and conventional culture systems for production of 10 mg recombinant protein.

Culture System	Culture volume per disposable (ml)	Max. protein X conc. (µg/ml)	Supernatant (ml) per 10 mg protein	Serum use (ml)	Number of harvests	Cultivation time (weeks)
CELLine *adhere* (1 x)	25	65.5	153	15 *	6	8
Non-compartmentalised e.g. Roller bottles (6 x)	350	4.4	2273	227	1	1

* An additional 48 ml serum is needed when supplementing the medium compartment with 2% FCS

Conclusion

The two-compartment bioreactor CELLine *adhere* has been specifically developed for high-density culture of anchorage dependent cells. In the presented study, adherent CHO cells were successfully cultivated for several weeks and reached a density of 1.6 x 10^7 cells/ml, which represents a 10-fold increase when compared to non-compartmentalised culture systems such as T-Flasks.

As a result of the high cell density, recombinant proteins expressed in CELLine *adhere* accumulate to high concentrations (in this study a 9-fold increase compared to conventional systems was achieved). Accordingly, the amount of disposables and the volume of supernatant that have to be handled decrease, the consumption of serum is significantly reduced and the down-stream purification process is simplified. Hence, CELLine *adhere* is ideal for research and small-scale manufacturing of recombinant proteins in anchorage-dependent cells.