

Long-Term High Level Protein Expression in Adherent, Protein-free Growing BHK Cells Using INTEGRA CELLine *adhere* 1000 Bioreactor Flasks

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Introduction

During the last decade the production of recombinant proteins and antibodies by mammalian cells has become a cutting edge technology. Compared to bacterial expression systems the products are correctly assembled, folded and glycosylated. Unfortunately the product yields in traditional cell culture are low so that the target proteins need to be captured from a large volume containing host cell proteins and often also serum components. While product contamination by serum can be overcome using protein-free medium, milligram-amounts of product proteins can only be obtained using small scale bioreactors or large numbers of Cell culture flasks and roller bottles. Such standard methods either require complicated equipment or are very time and disposal consuming. In addition, such methods are coupled with the need to handle large volumes in the downstream purification steps.

An alternative to this dilemma is the use of an easy to handle two compartment system which leads to high product concentrations. INTEGRA Biosciences has developed such a disposal flask-bioreactor for the production of recombinant proteins in a quasi-perfusion mode, in which cells are kept in a cell-compartment designed as a membrane bag, which is placed in a large bottle of medium. Cells are fed by the nutrients from the outer medium - compartment, which pass over the 10 KD-membrane while expressed target proteins are accumulated in the cell-compartment.

The following report evaluates the Integra Biosciences two compartment bioreactor, CELLine *adhere* 1000, ran in a semi-continuous mode over a 6 week period against a standard cell culture flask used in batch mode.

Material and Methods

The adherent BHK cell line SF-BHK SEAP adapted to growth in chemically defined protein-free MAM-PF4 medium (EugeneX Biotechnologies) expressing secreted alkaline phosphatase (SEAP) as a reporter-protein was used in this study. In batch mode the 15 ml cell compartment of the CELLine *adhere* 1000 flask was inoculated with 1.4 million cells per ml. The medium compartment was filled with 500 ml MAM-PF4 medium and fed at day 5 with additional 500 ml fresh medium.

After obtaining the metabolic data of the cells in this environment we started the semi-continuous run with 1.9 million cells per ml in the cell-compartment and with 1000 ml MAM-PF4 medium in the medium-compartment. Sampling was done daily in the batch mode or at day 4 and together with the harvest at day 7 in the semi-continuous mode. Samples from the cell-compartment were obtained by careful removal of the total volume (15 ml) out of the bag. A 1.5 ml aliquot was then used for cell counts and analytical assays. The remaining volume was filled up to 15 ml with fresh medium

and returned to the cell-compartment. The samples were assayed for suspended living and dead cells by an automatic video-based cell counter (Cedex, Inovartis AG).

The concentration of target protein (SEAP) was measured by a photometric assay with samples from the cell compartment. Due to the permeability of the membrane for small molecules the metabolic parameters like glucose, lactate and ammonia were monitored in samples from the medium-compartment and led to the same values as samples taken from the cell compartment.

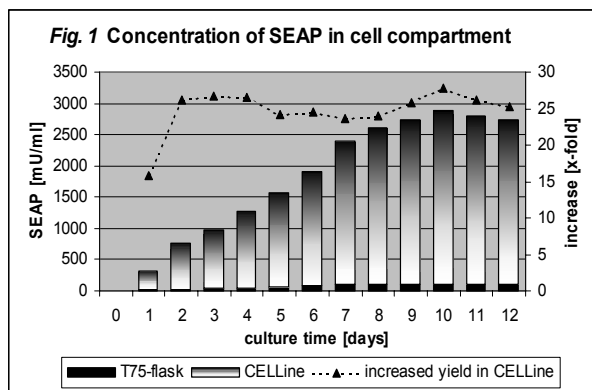
Results

Batch Cultivation

In a first batch-mode evaluation it was found that no SEAP reporter protein could be detected in the medium-compartment which means that the 10 kD-membrane retains all of the produced protein in the cell-compartment. In the 12 day batch cultivation of SF-BHK SEAP in the CELLine *adhere* bioreactor maximal product concentrations of 2700 mU SEAP

per ml where detected (Fig. 1). Compared to the traditional 75 cm² cell culture flask containing the same volume of medium and inoculated with the same cell density we found an average 25-fold increase of SEAP concentration in the cell-compartment of the CELLLine *adhere* bioreactor. While SEAP production increases steadily over the first 10 days, glucose uptake and the viability of cells in the supernatant decrease from day 8. Therefore, in our hands an ideal harvest point would be day 7 where no limitations on cell growth could be detected.

Due to the fact that SEAP-production is coupled to cell number and together with the cell counts of a suspended cell line in preliminary experiments it can be said that the cell number in the cell-compartment was about 25-fold higher compared with cell culture flask cultivation in the same volume.

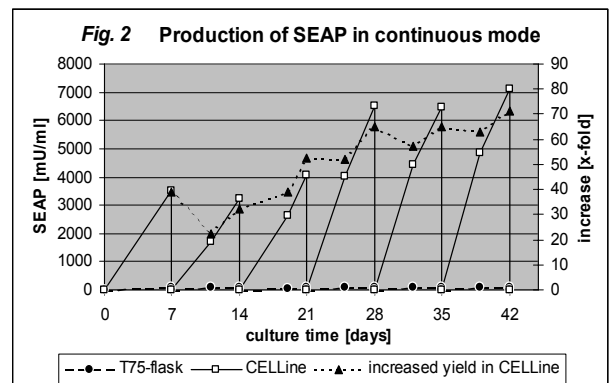


Semi-Continuous Cultivation

The long-term cultivation of BHK cells in CELLLine *adhere* was inoculated with a seeding density of 1.9 million cells per ml and was grown up without any sampling for one week to enable optimal attachment of the cells. The medium was changed and the cell-compartment was harvested once a week to reach an equilibrium at the maximal cell densities and to obtain maximal product yield (Fig.2).

In the attachment phase cell counts in the supernatant increase from nearly zero to a stabilized value of $1.3 \cdot 10^7$ cells per ml which suggests that the cell compartment is confluent with cells. The average viability of cells found in supernatant found an equilibrium between 40 and 50 percent. The determined cell numbers and viabilities represent only the non-attached living cells and dead cells, which detached from the surface or died in suspension. Therefore for adherent growing cell lines this parameter do not mirror the real cell numbers or viability in the cell compartment which must be several magnitudes higher.

A glucose uptake rate of 3 gram per week could be found in both CELLLine *adhere* and cell culture flask cultivation but the lactate formation was up to two times higher in cell culture flask than in the CELLLine *adhere*. This finding indicates that the oxygen transfer in CELLLine *adhere* is much higher than in cell culture flasks, suggesting a better metabolization of glucose in the CELLLine *adhere* bioreactor.



By this semi-continuous cultivation strategy a product concentration of 7100 mU SEAP per ml could be reached in a CELLLine *adhere* 1000 within a one week harvesting period (Fig. 2). In this experimental setup, to reach the same amount of recombinant protein by conventional cultivation methods approximately seventy 75 cm² cell culture flasks were necessary.

Conclusion

The two-compartment design of the CELLLine *adhere* bioreactor enables higher cell densities and corresponding product concentrations in batch and semi-continuous culture modes. Up to 70 cell culture flasks 75 cm² were necessary to achieve the same protein yields as that produced with a single CELLLine *adhere* 1000. The smaller volume of concentrated protein product also offers significant savings in time and material costs of the downstream processing and purification effort.

The relatively low cost and easy handling of the CELLLine *adhere* bioreactor, combined with its ability to be continuously harvested for several weeks, make for considerable savings in both material and labour processing costs. The minimised handling also significantly reduces the possibility of contamination.

Thus, the higher cell densities and viability and the dramatically higher protein yields achievable, combined with the low investment costs of this disposable bioreactor makes CELLLine *adhere* the system of choice for laboratory scale production of recombinant proteins.