Application Note

Cultivation of Suspension and Hybridoma Cells in CELLSTAR® CELLreactor™ Tubes

greiner bio-one

1. Introduction

Transfected mammalian cells in shaken suspension culture are widely used in biomedical research and pharmaceutical industry to produce recombinant proteins or antibodies[1]. Vast numbers of cells that are necessary to achieve significant amounts of protein are cultivated in large-scale bioreactors containing several to thousands of liters of culture volume.

For bioprocess development, optimisations of cell culture parameters such as medium composition are essential[2]. These optimisations can be laborious and expensive with the commonly used stainless steel or glass bioreactors. This is partly due to the large volume of these devices, mandating both high reagent consumption and high demand on facility space. Furthermore, the expense to clean and maintain large-scale bioreactors can be significant.
Therefore, bioreactors in milliliter scale that can be used as a disposable have become increasingly popular for the small-scale optimisation of process conditions. Among them, shake flasks are well established, however, their geometry and size prohibit efficient utilisation of incubator space. In addition, harvest of the cells by centrifugation poses a potential risk of contamination during the necessary additional handling steps.

A more favourable alternative are CELLSTAR® CELLreactor™ 15 ml und 50 ml tubes that can be placed at high density within a rack. The rack can be used with an orbital shaker (see Box 1) integrated with the incubator system or placed within as a stand-alone device. This allows for a very efficient parallelisation of experiments that is completed by the fact that CELLreactor™ tubes can readily spun down in commonly available centrifuges to enable rapid and secure cell harvesting.

Another important aspect of bioreactors is their ability to support aeration of the cell suspension. The CO_2 / bicarbonate buffering system is widely used to enhance the inherent buffering capacity of cell culture media. It relies on the dissolving of external CO_2 derived from the incubator atmosphere into the medium of the culture vessel. Therefore, sufficient aeration of the vessel is a prerequisite.

The abovementioned design properties of CELLreactor™ 15 ml or 50 ml tubes are completed with a filter screw cap for optimal ventilation of the tubes (Figure 1a and 1b). The filter membrane consists of a patented hydrophobic capillary pore membrane. The defined and constant pore size of 0.2 µm is achieved with minimal variation resulting in both optimal protection against contamination and efficient gas exchange. Thus, CELLreactor™ tubes are suitable for the small-scale cultivation of suspension cells and aerobic microorganisms, such as bacteria or yeast.

In this application note, CELLreactor™ properties with regard to gas permeability and the culture of suspension cells are presented.

Figure 1a: CELLSTAR® CELLreactor™ tubes are available as 15 ml and 50 ml tubes with filter cap.

Figure 1b: CELLSTAR® CELLreactor™ tube caps which feature 8 (50 ml tube) or 6 (15 ml tube) openings for excellent gas exchange.

Box 1: Orbital shakers are available with different agitation diameters up to 50 mm and for use at different operating conditions (i.e. atmospheres). They come as stand-alone devices or integrated incubator solutions.
Suppliers include Infors AG, Kühner AG, New Brunswick Scientific, Thermo Scientific and VWR.

2. Material and Methods

2.1 Cell Culture

Jurkat cells (DSMZ, Braunschweig, Germany) were cultivated in 90 % RPMI1640 (Biochrom AG, Berlin, Germany) + 10 % fetal calf serum (Biowest, Nuaillé, France) + 2 mM L-glutamine (Biochrom).
YL1/2 cells (ECACC, Salisbury, UK) were cultivated in DMEM (Biochrom), ISF-1 (Biochrom) or BD Cell™ MAb (BD, Darmstadt, Germany). Each of these media was supplemented with 10 % fetal calf serum + 2 mM L-glutamine. Cell cultures were carried out in cell culture incubators at standard conditions (37 °C, 90 % humidity, 5 % CO_2).
A Titramax 100 (Heidolph, Schwabach, Germany) plate shaker or a Mini Shaker (VWR, Bruchsal, Germany) were used as indicated.
For both cell lines, initial cell density was 10^5 cells/ml unless stated otherwise and they were passaged at 80 % confluency. Viable cell numbers were determined by staining cells with trypan blue and counting both live and dead cells.

2.2 Determination of pH kinetics

600 ml of DMEM medium were equilibrated to ambient level CO_2 overnight at room temperature. 20 ml of equilibrated DMEM medium were filled into each of ten CELLreactor™ 50 ml tubes, five 50 ml tubes with tightly closed standard cap, five 50 ml tubes with loosened standard cap and five 75 cm^2 suspension culture flasks with filter cap. The vessels were placed on an orbital plate shaker at 400 rpm in a standard incubator at 37 °C, 90 % humidity and 5 % CO_2. The pH in one tube of each group was determined hourly with a pH electrode. The respective tubes were discarded after each pH measurement.

2.3 Determination of evaporation

Cap wells of CELLreactor™ tubes were sealed with adhesive tape as indicated. These tubes as well as 50 ml tubes with tightly closed standard cap, 50 ml tubes with loosened standard cap and 25 cm^2 suspension cell culture flasks were filled with 10 ml of water as indicated. The vessels were placed on an orbital plate shaker at 400 rpm into a standard incubator at 37 °C, 90 % humidity and 5 % CO_2.
The mass of each of the vessels was determined daily. Considering that the water density is 1 kg/cm^3, daily loss of water was calculated in µl/h.

2.4 Dot Blot

To detect anti-tubulin antibody, nitrocellulose membrane was soaked in Tris-buffered saline (TBS) for 10 min. 100 µl aliquots of the respective cell culture supernatant were loaded onto the nitrocellulose membrane using a Dot Blot manifold.
Samples were air-dried on the membrane for 60 min and then washed with 100 µl of TBS containing 0.1 % Tween-20 (TBS-T).
The membrane was removed from the apparatus and nonspecific binding sites were blocked overnight at 4 °C with 5 % skimmed milk powder in TBS-T. After washing the membrane twice with TBS-T and another two times with TBS, the bound antibodies were detected with alkaline phosphatase-conjugated donkey anti-rat immunoglobulins (Dako, Hamburg, Germany), followed by colorimetric reaction with BCIP/NBT substrate (Roche, Mannheim, Germany).

3. Results and Discussion

To characterise some basic features of the CELLreactor™ 50 ml tube, the following properties were evaluated:
- CO_2 permeability
- Water vapor evaporation
- Suitability for the culture of suspension cells
- Suitability for biotechnological applications

3.1 CO_2 permeability

By placing cell culture medium containing bicarbonate (such as RPMI 1640 or DMEM) outside the CO_2 incubator at ambient level CO_2 (= 0.04 %), an alkalisation of the medium takes place. This is a typical scenario that occurs with cell culture vessel handling steps outside the incubator. If medium alkalisation takes place, it is important to achieve the desired physiological pH in the medium as fast as possible after returning the culture vessel to the CO_2 incubator.

Figure 2 depicts the performance of CELLreactor™ tubes with regard to CO_2 input after alkalisation of RPMI 1640 (top) or DMEM (bottom) medium. With both media, the courses of pH adaption were similar. As expected, the pH of the two media stayed alkaline in 50 ml tubes with a tightly closed standard cap, indicating gas-tightness of this set-up.

In shaken tubes with standard cap and in stationary flasks with a filter cap, complete pH adaption was achieved within 3 hours from placing them in the incubator. Although the liquid-air interface is smaller in the tube compared to the flask, shaking of the tube was sufficient to compensate for this disadvantage.

The pH adaption was more rapidly achieved already after 2 hours with medium placed in CELLreactor™ tubes agitated. The fact that pH adaption occurs fairly slowly in stationary tubes indicates that shaken culture conditions are necessary for efficient aeration if tubes are used in upright position.

This emphasises that CELLreactor™ tubes are very efficient in aeration properties due to the excellent gas exchange properties of the filter membrane used together with shaking culture conditions.

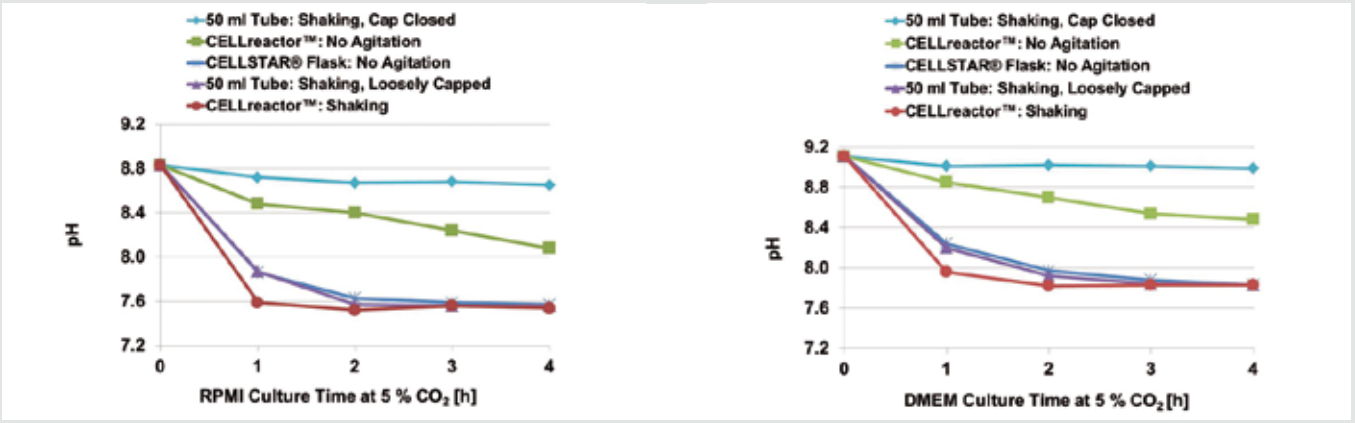

Figure 2: pH kinetics of 20 ml RPMI 1640 (left) or DMEM (right) medium after alkalisation. Indicated conditions were applied in 5 % CO_2 atmosphere at 37 °C. Tubes were agitated on an orbital plate shaker at 400 rpm.

3.2 Water vapor evaporation

An inherent feature of filter membranes is their water vapor permeability. Therefore, the effect of closing individual cap openings on sample evaporation was investigated.

Figure 3 shows the evaporation rate in different vessels containing 10 ml of water. With CELLreactor™ tubes with none of the eight openings sealed, an evaporation of 4 µl/h was detectable. CELLreactor™ tubes featuring four sealed and four non-sealed openings in their filter cap exhibit a comparable evaporation to 50 ml suspension cell culture flasks with a filter cap.

Only one ventilation opening in the CELLreactor™ cap leads to a similar evaporation as the 50 ml tube with loosened standard cap. No evaporation is detectable for the 50 ml tube with a tightly closed standard cap.

In conclusion, evaporation can be regulated by closing individual cap openings of CELLreactor™ tubes. This function allows for balancing the need for efficient aeration and the demand to prevent evaporation.

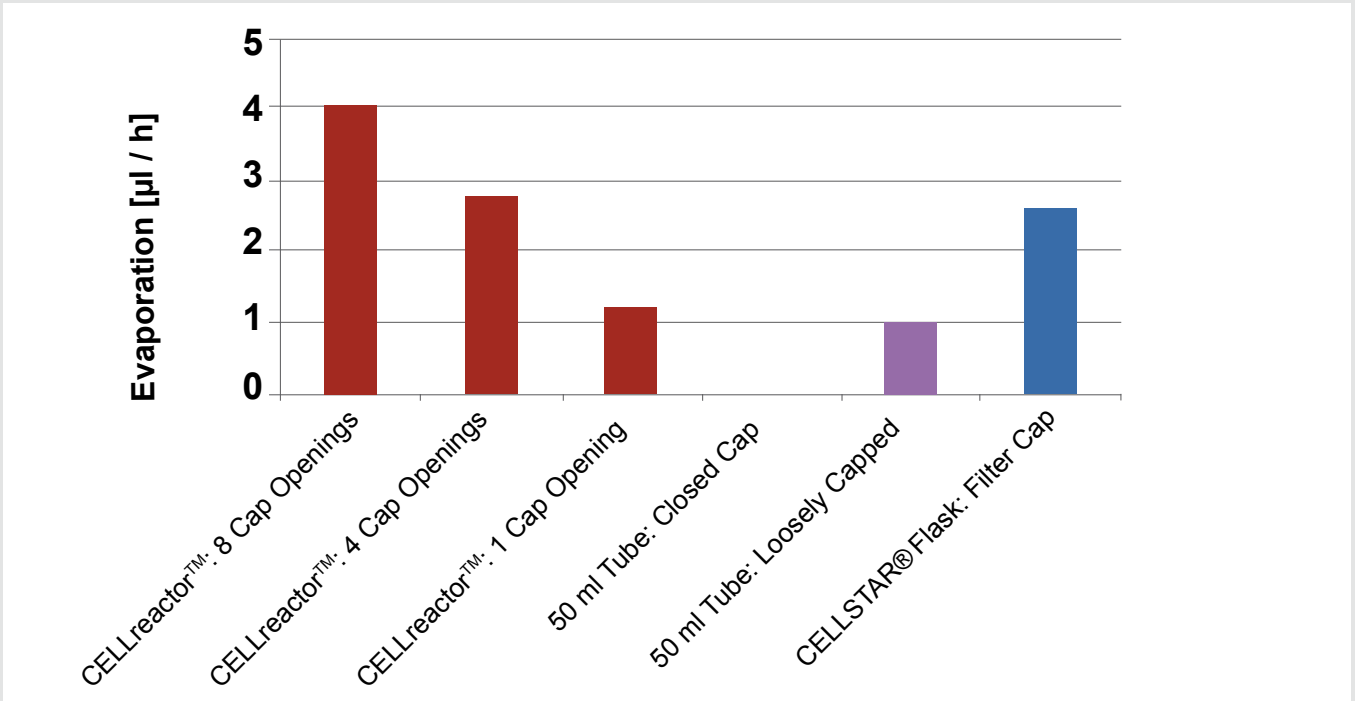

Figure 3: Average evaporation rate [µl/h] of different vessels containing 10 ml of water. Tubes were agitated on an orbital plate shaker at 400 rpm.

3.3 Suitability for the culture of suspension cells

One advantage of using CELLreactor™ tubes is the ability to cultivate and harvest cells in the very same tube. To demonstrate the feasibility of cultivating suspension cells in the CELLreactor™ tube, 1 x 10^5 Jurkat cells in 20 ml medium were seeded into CELLreactor™ 50 ml tubes or standard 250 ml CELLSTAR® suspension culture flasks with filter cap. Both CELLreactor™ tubes and flasks were cultivated lying in the incubator without any agitation.

Figure 4 shows that cell growth in CELLreactor™ tubes is comparable to cell proliferation in standard flasks. The number of viable cells increases steadily over the course of the culture for four days to approximately 10 x 10^5 cells in both vessels.
In contrast to the culture in flasks, CELLreactor™ tubes enable harvest of the cells by centrifugation without any further handling steps.
Thus, CELLreactor™ tubes present an interesting option for efficient, time-saving suspension cell culture with minimised contamination risk.

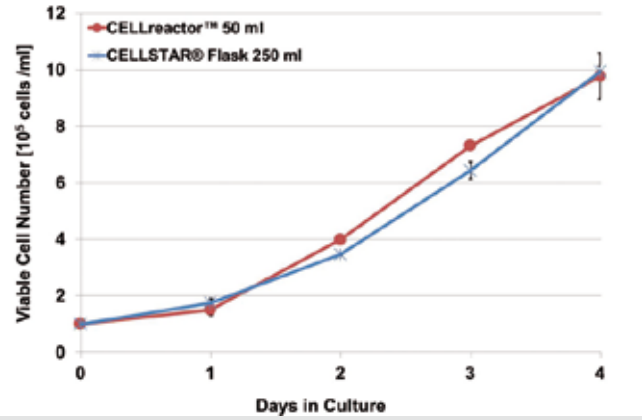

Figure 4: Culture of Jurkat cells in 20 ml medium without agitation.

3.4 Suitability for biotechnological applications

To test the suitability of CELLreactor™ 50 ml tubes for biotechnological applications such as antibody or protein production, YL1/2 hybridoma cells were cultivated with different media. These cells are adapted to shaken suspension culture and produce rat anti-tubulin antibodies that can be harvested from the culture medium supernatant.

Cells were seeded at 0.5 x 10^5 cells / ml in 20 ml of DMEM, ISF-1 or BD Cell™ MAb medium and placed on an orbital shaker at 200 rpm. After four days, cells grown in DMEM medium achieved a viable cell number of 7.5 x 10^5 cells / ml (Figure 5, top) with a continous cell viability of more than 90 % (Figure 5, bottom). In contrast, cells grown in ISF-1 or BD Cell™ MAb media did not exhibit significant cell proliferation and the cell viability was lower than 80 %.

The significance of this finding is confirmed by dot blot analysis of the media supernatants (Figure 6). Only cells cultured in DMEM medium secreted a significant amount of anti-tubulin antibody into the medium.
This highlights that small-scale experiments requiring a minimum amount of space can be carried out with CELLreactor™ tubes to optimise cultivation conditions.

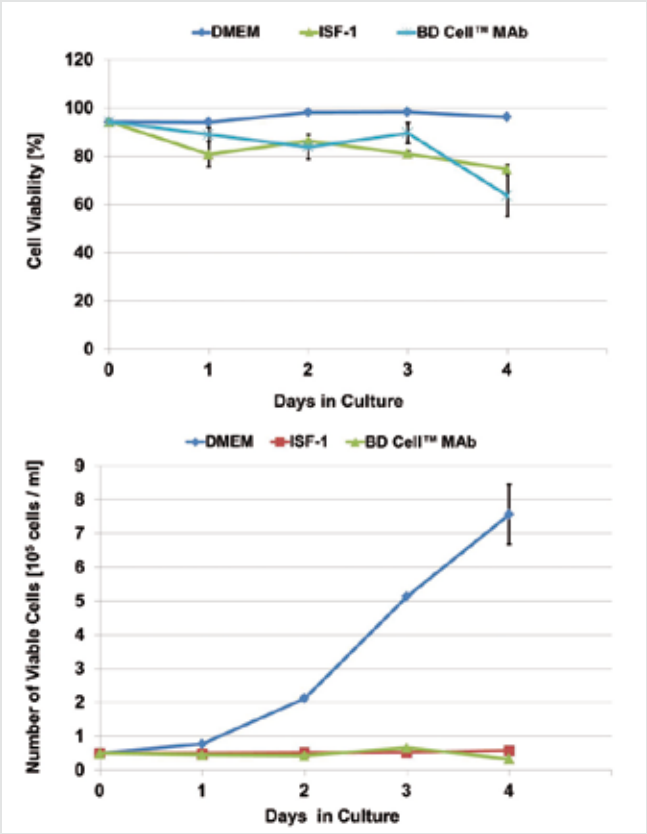

Figure 5: Suspension culture of antibody-producing YL1/2 hybridoma cells in CELLreactor™ 50 ml tubes for 4 days with 20 ml medium. Tubes were agitated at 200 rpm on an orbital shaker.

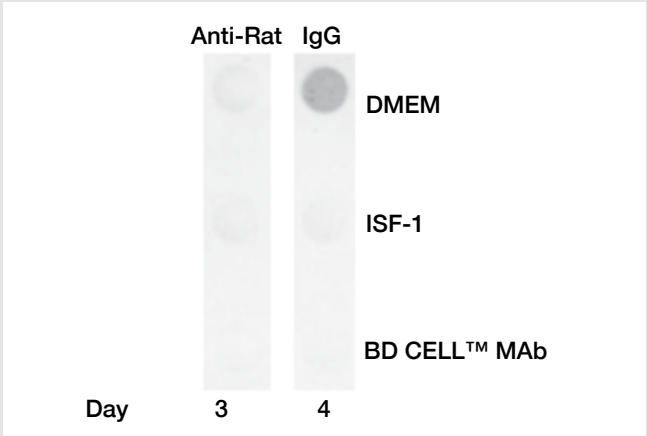

Figure 6: Dot Blots for anti-tubulin antibodies derived from different medium supernatants after 3 and 4 days of YL1/2 cell culture.

4. Conclusion

The results portray the excellent suitability of CELLreactor™ tubes for the culture of suspension cells. By agitating the cells on an orbital shaker without any internal stirrers, disadvantageous foam formation and shear stress can be eliminated. The filter cap prevents contaminations from entering the tube interior without hampering the aeration of the tube.

Moreover, CELLreactor™ tubes can be used as small bioreactors for the optimisation of culture parameters in a biotechnological setting. Their geometry allows for convenient multi-parametric experiments when implemented within tube racks.

5. Acknowledgement

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6. References

1. Matasci, M., Hacker, D. L., Baldi, L. & Wurm, F. M. Recombinant therapeutic protein production in cultivated mammalian cells: current status and future prospects. Drug Discov. Today Technol. 5, e37–e42 (2008)

2. Wurm, F. M. Production of recombinant protein therapeutics in cultivated mammalian cells. Nat. Biotechnol. 22, 1393–1398 (2004)

Ordering Information

Cat.No.	Product Description	Quantitiy per Bag	Quantity per Case
188 240	CELLSTAR® CELLreactor™ Filter Top Tube, PP, 15 ml, sterile	20	300
227 245	CELLSTAR® CELLreactor™ Filter Top Tube, PP, 50 ml, sterile	20	500

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