Efficiency in antibody production: a comparison between Cellab® Bioreactor System with hollow fiber modules, miniPERM® bioreactor and T-flask

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In vitro cell culture systems for high-density cell growth are very important for research and industrial applications. Cellab GmbH has developed a new semi-automated system for high cell density culture, called Cellab® Bioreactor System. This hollow fiber bioreactor system is compared with the miniPERM® bioreactor and a standard T-flask for the production of monoclonal antibodies.

Materials and Methods

Cell culture systems: Cellab® Bioreactor System with hollow fiber bioreactors, miniPERM® bioreactor and T-flask

Cellab® Bioreactor System is a standardized disposable bioreactor system for cell cultivation for various applications e.g. the production of monoclonal antibodies. The system consists of the Cellab® Docking Station and the Cellab® Disposable Set. It can be operated in a standard CO₂ incubator (Fig. 1). Cell culture medium is enriched with oxygen by a gas transfer module and circulates from a medium bag through a closed tube system into the bioreactors. The system feeds the cells with nutrients and eliminates metabolic waste automatically. The Cellab® Disposable Set is equipped with two mid-sized hollow fiber bioreactors. This type of bioreactor is a two-compartment-system which separates the cells from the medium by a semipermeable membrane (Fig. 2A). Hollow fibers are fitted into a cylindrical cartridge (150mm in length x 9mm inner diameter). The fiber wall is functional as an ultrafiltration membrane with a molecular weight cut-off (MWCO) of 20KDa. Cells are grown in a volume of 5ml in the space surrounding the fibers (ECS; extra capillary space). Cell culture medium is pumped through the hollow-fiber lumen (ICS; intra capillary space). Nutrients, metabolites and gases can diffuse through the semi-permeable membrane; the antibodies produced are retained in the ECS cell culture compartment (Fig. 2B).

Figure 1: Cellab® Docking Stations with Cellab® Disposable Sets in a standard CO₂ incubator

Figure 2A: Hollow fiber bioreactor design shows medium flow through the ICS.

Figure 2B: Cross section of hollow fiber (ID 200 µm, OD 280 µm) illustrates exchange of nutrients and metabolites during cell culture. Scale bar: 50 µm.
The miniPERM® bioreactor (Sarstedt AG & Co.) is a cell culture system in which the cell compartment is separated from the nutrient medium compartment by a flat dialysis membrane with a MWCO of 12.5KDa. The nutrient compartment can hold 350ml of medium and the cell compartment has a volume of 35ml. In the miniPERM® bioreactor system the bioreactor is rolled on a roller which fits into a standard incubator.

A standard T75-flask from TPP® Switzerland was also used in this comparison.

Cell culture

Two monoclonal antibody secreting hybridoma cell lines were used to compare cell culture and antibody production efficiency of the three cell culture systems. The hybridoma cell line MFP is a high antibody producer cell line. In total 12.0x10^6 cells (in DMEM plus 10% FCS) were inoculated into the ECS of one of the two hollow fiber bioreactors of the Cellab® Bioreactor System. The second hybridoma cell line Ata is a low antibody producer cell line. In total 5.0x10^6 Ata cells (in DMEM plus 10% FCS) were inoculated into the ECS of the second hollow fiber bioreactor of the Cellab® Disposable Set. Both cell lines were also cultured in the cell compartments of two miniPERM® bioreactors. The medium bag of Cellab® Bioreactor System was filled with 1.5l culture medium (DMEM plus peptone) and the nutrient compartment of the miniPERM® bioreactor was filled with 350ml DMEM plus peptone.

Culture in the T75-flasks was started with 1.2x10^6 cells/ml (DMEM plus 10% FCS) for the MFP high producer cells and 1.0x10^6 cells/ml (DMEM plus 10% FCS) for the Ata low produced cells. Every three to four days the cell culture was collected from the T-flasks and centrifuged. The culture supernatants, containing the monoclonal antibodies, were kept as harvest to determine the concentration of the produced antibodies. Part of the cells harvested were returned into the flasks and used for continuation of the culture.

For all systems cell culture was performed under standard conditions in a CO₂ incubator (37°C, 8% CO₂). The Cellab® Docking Station was adjusted to a continuous medium flow of 10ml per minute and a low gas flow (level 2). The exchange of 1.5l nutrient medium was performed manually using either by peristaltic pump or by gravity.

Sample collection

Sample collection was performed by taking 0.1 - 0.2ml aliquots from the ECS and the ICS of the two hollow fiber bioreactors of the Cellab® Disposable Set, from the cell and the nutrient supply compartments of the two miniPERM® bioreactors and from the T-flasks. For harvesting, the ECS of the hollow fiber bioreactor was flushed with 5ml DMEM. Antibody concentration was determined in all fractions.

Cell metabolism and antibody determination

Glucose consumption was checked in the bioreactors, medium bag and nutrient compartment using a standard glucose measurement system GlucoCheck XL from Aktivmed GmbH. Medium was changed when glucose concentration fell below 200-300mg/dl.

Antibody concentration was measured by using single radial immunodiffusion according to Mancini (Mancini et. al., 1965). In addition, monoclonal antibody yield in the harvest samples was determined after purification of the antibodies by ProteinA affinity chromatography in a FPLC machine.
Results

In general in the dialysis membrane based systems, Cellab® Bioreactor System and miniPERM® bioreactor, much higher antibody yields were achieved than in T-flasks (Fig. 3).

For the MFP high antibody producer hybridoma cell line, the total amount of antibodies produced in the harvest from the miniPERM® bioreactor was 6 times higher and in the harvest from the Cellab® Bioreactor System was 10 times higher than in the T flasks. Comparison between the Cellab® Bioreactor System and miniPERM® bioreactor showed a 66% higher yield of antibodies produced in the Cellab® Bioreactor System.

With the Ata low antibody producer hybridoma cell line, the total amount of antibodies produced in the harvest from the miniPERM® bioreactor was 3 times higher and in the harvests of the Cellab® Bioreactor System 6 times higher than in the T flasks. Comparison between the miniPERM® bioreactor and the Cellab® Bioreactor System showed an 81% higher yield of antibodies produced in the Cellab® Bioreactor System.

In order to purify 25mg of monoclonal antibodies from the MFP cell line, only 5ml of Cellab® cell culture supernatant and 58ml miniPERM® bioreactor supernatant had to be processed. In contrast, processing of 2500ml from the T-flask was required to produce 25 mg of antibodies (Fig. 4).

The reason for the higher production of antibodies using the Cellab® Bioreactor System is the increased ratio between the volume of the cell compartment (ECS) and the medium compartment (ICS) - 1:200 compared to 1:10 in the miniPERM® bioreactor. The optimized supply of medium to the cells and the optimized elimination of metabolites through the hollow fiber 20KDa MWCO membranes is an additional reason.

The results have demonstrated that the Cellab® Bioreactor System equipped with hollow fiber bioreactors is a preferred system for short-term and long-term cultivation of hybridoma cells for the production of monoclonal antibodies.
Figure 3: Total amounts of monoclonal antibodies purified from the harvests of MFP (high antibody producer) and Ata (low antibody producer) cell lines after a cultivation period of 12 days.

Figure 4: Volume of cell culture supernatant that has to be processed to obtain 25mg of purified monoclonal antibodies from MFP (high antibody producer) and from Ata (low antibody producer).