

Growing potential: mAb production with Fibra-Cel

BIOMANUFACTURING The market for monoclonal antibodies continues to grow steadily, but there are still bottlenecks in the development of new products, including long development times mostly due to R&D. Advanced bioprocess equipment that meets the specific demands of mAb-producing hybridoma can accelerate design and production, and reduce overall development costs along the way.

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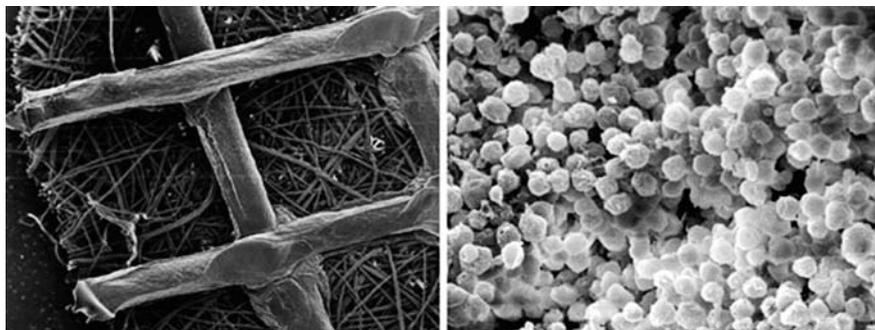


Fig. 1: Scanning electron micrograph of Fibra-Cel disks (left); mouse-mouse hybridoma DA4.4 immobilized on Fibra-Cel disks during production at 1×10^8 cells/cm³ of packed-bed volume (right).

Recent years have seen a continuous rise in the market for monoclonal antibody (mAb)-based therapeutics, diagnostics and imaging modalities. In particular, mouse mAbs produced in hybridoma cells are experiencing a resurgence in growth because of the increasing demand for immunodiagnostic tests, which identify circulating tumor cells, stem cells and pathogens. For example, the diagnostic used to differentiate between leukemia subtypes employs hybridoma-generated mAbs to detect B and T cell subsets. Another imaging technique employed in the diagnosis of prostate cancer hinges on a mAb specific for a human prostate cancer cell surface marker which was created using hybridoma technology. In ad-

dition, common pregnancy tests detect the condition with the help of mAbs specific to the β -chain of the pregnancy hormone hCG. This growing market segment is predicted to reach US\$19.83bn by 2015, and is expected to continue to expand throughout the next decade.

The effective hybridoma

The most common method of producing mAbs for diagnostics and imaging in the biopharmaceutical industries is hybridoma technology. Hybridomas are hybrid cell lines generated from fusing a B cell producing an epitope-specific antibody with a myeloma cell carrying the ability to grow in cell culture and lacking antibody chain synthesis.

Stirred-tank bioreactors are often used in the large-scale production of hybridoma-derived diagnostic mAbs. Besides the culture volume, the advantage of bioreactors compared to conventional cell culture methods is the automated and precise control of all important culture conditions and process parameters.

Proven technology with scale-up potential

The Fibra-Cel® technology has been established as an excellent method for the growth of suspension and anchorage-dependent cell lines. The three dimensional structure of the Fibra-Cel disk provides an excellent solid-support matrix for the entrapment or attachment of animal cells, allowing constant perfusion of nutrients in a low-shear environment. It is used predominantly in perfusion processes for the production of secreted products such as recombinant proteins and viruses. Since the 1980s, scientists around the globe have been using Fibra-Cel to grow a wide range of mammalian and insect cell lines. Recently it was also shown that hybridoma cells such as DADA4.4, 123A, 127A, GAMMA, 67-9-B can be successfully cultivated on Fibra-Cel disks at high cell densities (see Fig. 1). By im-

proving cell densities, the mAb titers in production processes can be massively increased.

Originally used in autoclavable CelliGen® cell culture bioreactors (Eppendorf), Fibra-Cel technology has now been successfully adapted to sterilizable-in-place systems as large as 150 liters, allowing for seamless scale-up to commercial production. With the BioBLU® packed-bed, single-use vessels (Eppendorf), Fibra-Cel technology is also available for those who prefer the advantages of disposable systems.

Increasing productivity fivefold

Scientists at the Eppendorf Research & Development Lab in Enfield, CT, USA have been evaluating DA4.4 hybridoma cell cultures on Fibra-Cel disks. To demonstrate that the proprietary packed-bed basket impeller is capa-

ble of robust, reproducible high-density hybridoma culture under perfusion conditions, two independent trials were conducted using the suspension-adapted DA4.4 hybridoma cell line in a CelliGen 310 bioreactor (Fig. 2). This packed-bed impeller creates a low differential pressure at the base of the impeller tube, which circulates the medium throughout the system. The medium receives gases through a sparger located at the bottom of the inner tube, protecting the cells from exposure to the gas liquid interface. This results in low turbulence and low shear stress on the culture.

In preparing the inoculum, DA4.4 hybridoma cells were grown in 1 L shake flasks at 37 °C with 5% O₂ and agitation set at 95 rpm. The culture medium was prepared using Gibco® Hybridoma-SFM complete DPM powder supplemented with 5% Hyclone® Fetal Bovine Serum and 1% Gibco liquid

Pen/Strep. For bioreactor cultivation, the 1.75 L vessel working volume was inoculated with a target total of 4.1 x 10⁸ cells. Actual viable cell numbers were 3.5 x 10⁸ cells (2.2 x 10⁵ cells/mL) for the first run and 4.8 x 10⁸ cells (3 x 10⁵ cells/mL) for the second run. For both runs, hybridoma cells were cultured in CelliGen 310 bioreactors for nine consecutive days, using the basket impeller system packed with 75 g of Fibra-Cel disks. Perfusion was initiated for each bioreactor on day 3 and continued through day 9. Initially, the main objective was to increase the perfusion rate to maintain a glucose concentration at or above 1 g/L. For the second bioreactor experiment, the perfusion rate was adjusted to match the first bioreactor rate in order to make the two runs as similar as possible. Daily off-line measurements of glucose concentration were performed from both bioreactors, and the glucose consumption



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Cultivation Setpoints	
Agitation	80 rpm
Temperature	37 °C
pH	7.15
DO	50 %
Gas supply	CO ₂ for pH control
Gas flow conditions	0.4 SLPM
Vessel	1 L glass water jacketed
Fibra-Cel	75 g

Day	Perfusion volume (L)
3	0.73
4	1.81
5	4.25
6	5.50
7	4.25
8	4.75
9	5.00

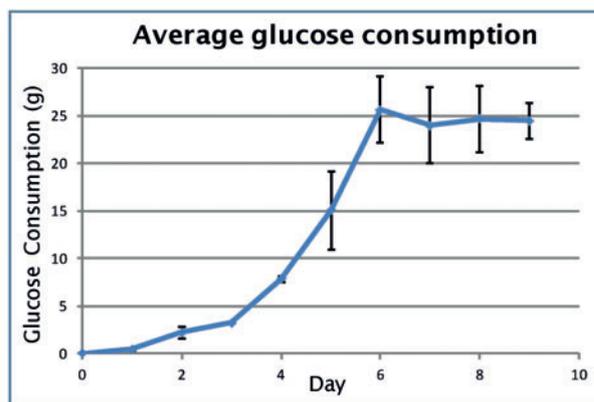


Fig. 2: Experimental setup and results; cultivation parameters, perfusion volumes and equipment used (left and upper right). Average glucose consumption of both runs as an indicator of cell productivity (lower right). Error bars indicate standard error of the mean.

was calculated for each time point and plotted as an average of the two independent runs.

The performed experiments have demonstrated that the implementation of packed-bed Fibra-Cel growth conditions in addition to perfusion production methods greatly increase yields of hybridoma cells, which are inherently sensitive to waste buildup. Fig. 2 shows the rate of glucose consumption across both trials. Comparable consumption was observed, indicating reproducible growth performance of hybridoma cells in this environment. One conclusion is that the use of Fibra-Cel in the basket impeller system on the CelliGen 310 is an excellent method for high-density hybridoma culture. In batch runs with common pitched blade impellers, hybridoma cells usually peak at approximately 5 g/day of glucose

consumption (data not shown). The packed-bed basket impeller system presents significantly higher productivity, with glucose consumption peaking at an average 25 g/day. In addition, if growth conditions are maintained by continued fresh media perfusion and glucose concentration is never allowed to fall below 1 g/L, hybridoma can be continuously cultured in the basket many days after the nine-day window observed in this study. This further increases productivity and decreases overall antibody production costs. No optimisation of growth conditions were attempted for either bioreactor run.

Conclusions

In summary, Fibra-Cel provides benefits in research laboratories as well as for commercial production of mAbs.

Because higher yields can be achieved, smaller bioreactors can be used to substantially reduce initial capital expenditure, as well as to reduce the utilities required for operation (such as electricity, water, and steam if required). In addition, because the cells remain entrapped, the packed bed eliminates the need for cell filtration to separate cells from the end product, thus simplifying harvesting. Finally, product recovery and downstream processing can be more easily controlled because users can determine the volume of harvest material that is to be processed at any given time.

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