Cytokine Production using miniPERM® Bioreactor

miniPERM® Bioreactor

* **arium 611VF, recommended for application**
Type I water purification system for low TOC and low endotoxin applications. Water quality to 18.2 MΩ × cm, endotoxin levels <0.001 EU/ml, TOC level <1 ppb, product flow rate up to 1.5 l/min.

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Introduction
The miniPERM Bioreactor was originally conceived and developed as a culture system for growing hybridoma cells to high densities and for the production of monoclonal antibodies in high concentrations. As a consequence of the miniPERM special design and construction it was, in most, cases possible to achieve and in many cases to exceed the original goals of:

- cell densities greater than 10×10^6 cells/ml
- antibody concentrations greater than 1.0 mg/ml

It soon became apparent that this newly developed small bioreactor was capable of far more than had originally been foreseen. Not only hybridoma cells but also plant cells, insect cells, tumour cells and animal somatic cells could be cultivated to high densities in the miniPERM. It was, therefore, natural to apply this cultivation method to another type of cells with great significance both for biological and medical research: genetically engineered cells. More specifically, to cultivate tumour cells that have been transfected with genes encoding potent biological compounds for the purpose of obtaining these compounds.

In contrast to hybridoma cells, genetically transfected cells often produce the desired gene product in very small amounts. Columbo et.al. (1) report production rates ranging from a few picograms to several nanograms per 10^6 cells per 24 hrs. This is markedly lower than those described for mouse hybridoma cells which are in the order of several micrograms per 10^6 cells per 24 hrs (Fazekas de St. Groth, 2).

Our goal was to culture cytokine gene transfected cells that had previously been produced and described by others and to achieve, in the miniPERM Bioreactor, higher concentrations of cytokines than is posssible with the usual stationary culture methods. For this purpose we obtained cytokine tranfected cell lines from various research groups. These transfectants had been derived from the cell lines X63, J558, P815, C26 and B16 and produce the cytokines IL-2, IL-4, IL-7, IFNg, G-CSF and GM-CSF, respectively.
We present here the first report of our work with these cells.

Materials and Methods
Transfectants were grown in culture media (usually RPMI) containing 10% FCS and the selective agent neomycin certain cases geniticin.
The use of ultrapure* water is strongly recommended when rehydrating powdered culture media and additives.
Stationary cultures were expanded to a density of approximately 2×10^6 cells, then transferred to the production module of the miniPERM.
Various additional supplements were added to the culture media as required by each cell type. Transfectants derived from X63 cells were particularly sensitive to the composition of the culture media and required extensive supplementation (glutamine, glucose, NCTC-109, exalacetate, sodium pyruvate, insulin, mercapto-ethanol, Pluronic F-68, Primatone RL, Penicillin and Streptomycin) for growth in the miniPERM.
At the other extreme, the P815 based transfectants could be grown as described by the authors, in DMEM supplemented with 10% FCS, in both the expansion and the production phases.

The cytokine concentration in the culture media was ascertained using biological tests. In the case of IL-2, concentrations were determined both by a test of biological activity using the cell line (CTLL-2) (3) and by an indirect ELISA using the monoclonal antibody D10. A hybridoma cell line producing this antibody was kindly provided by A. Schimpl of the Institute of Virology and Immunology in Würzburg.

Results

Four attempts to culture tranfectants based on X63 cells, received from the Basel Institute for Immunology (4), have thus far proved unsatisfactory. These cells are exceedingly demanding and the optimal growth conditions for these cells in the miniPERM have not yet been determined. In most cases the cells achieve a density of 20×10^6 cells/ml and then, in spite of multiple media changes, die-off several days later.

Using J558 based transfectants, for example J558-IL-4 received from Dr. Diamantstein (5), we observed a rapid increase in cell density from 2×10^6 to $60–70 \times 10^6$ within a few days. These cells also die-off within the ensuing days although not as rapidly as the X63 transfectants. A 10–20 fold increase in cytokine production was achieved during our trials, however, this result falls short of the predictions based on the increase in cell density. The best results to date have been achieved with transfectants based on P815 obtained from Dr. Kourilsky (6).
We were able to maintain the P815-Il-2 cells at a density of $40–45 \times 10^6$ cells/ml for almost 2 weeks, albeit with an increasing proportion of dead cells.
After 13 days the culture medium contained 112,000 IU or approximately 6.0 micrograms IL-2 per ml. This is more than a 200 fold increase over the concentration of cytokine obtained from this cell line in stationary culture.

Discussion

One surprising result obtained in these studies was the high cell density reached with transfectants based on cells of the cell lines J558 and P815. Such high cell densities have never been reached with hybridoma cells grown in the miniPERM. This difference in obtainable cell densities may be due to the difference in cell size. The tumour cells used for transfection are smaller than hybridoma cells and therefore a greater number of cells would be required the achieve an equivalent biomass.

The low concentration of cytokines produced by S63 and J558 based transfectants are much less easily explained. It is possible that we have not yet discovered the optimal culture conditions for these cells in the miniPERM, or that the cytokines are destroyed in the culture media.
The high yield of cytokines obtained from P815-IL-2 transfectants, a yield which is significantly higher than the cell density would lead us to expect, can only be explained as the accumulation of the cytokine over the course of the production period. This explanation forces us to conclude that in spite of the high cell density and large numbers of dead cells, the secreted cytokine molecules are not destroyed in the culture, at least not in the case of cells of this lineage.

Conclusions

- It is possible to cultivate genetically transfected cells in the miniPERM bioreactor to high densities and to obtain the encoded gene products in high concentrations.
- The dialysis membrane in the standard model miniPERM with an MWCO of 12,5 kD allows the accumulation of IL-2, IL-4 and IL-7. The cytokines produced appear to be entirely within the production module since no cytokines could be detected in the media.
  For gene products of lower molecular weight it will, however, be necessary to employ membranes with lower MWCO values. Membranes with a MWCO of 3,5 kD are available and production modules fitted with such membranes are available by special order from In Vitro Systems & Services GmbH. However, membranes with a low MWCO will also inhibit the passage of nutrients and metabolites, resulting in culture conditions which are less optimal than those obtained using the standard membranes.
- Cytokines are not inactivated or destroyed under the high density culture conditions in the miniPERM, instead accumulate during the culture period, even in the presence of large numbers of dead cells.

Literature

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