Cell culture technology: hamster cells and the production of biopharmaceuticals

Biopharmaceuticals are one of the best selling drugs. The majority of successful biopharmaceutical ingredients are produced in CHO cell lines. Strong competition forces biopharmaceutical producers to continuously improve the drug development process and the production methods, which requires highly qualified experts.

Biopharmaceuticals are macromolecular substances (proteins, nucleic acids) that cannot be chemically synthesised. Instead, they are produced in genetically modified living cells. The first biopharmaceuticals to enter the international market such as insulin and growth hormone were produced in easy-to-cultivate cloned bacteria. Bacteria grow quickly and generate high quantities of recombinant protein. However, bacterial cells are unable to modify proteins, for example attach sugar chains (glycosylation), on the post-transcriptional level, something that human proteins are
able to do.

This is why more complex recombinant proteins are produced in yeast cells, which are relatively easy to cultivate at low cost. In addition, yeasts are eukaryotes and therefore able to carry out glycosylation and form disulphide bridges. However, the expression of proteins in yeast is more difficult to control than in bacteria and yeast-specific glycosylation patterns have been shown to differ from those of mammalian cells. This is why many of the most important modern biopharmaceuticals are produced in mammalian cell lines, despite the fact that cultivating them is more difficult and more expensive than bacterial cells. Moreover, in contrast to microorganisms, mammalian cell lines only produce small quantities of recombinant proteins. Biopharmaceuticals such as Enbrel, Remicade, Rituxan, Avastin, Herceptin and Humira achieve annual revenues of more than 5 billion US$, thus making their production in mammalian cells a profitable though highly competitive business in which all cost factors are meticulously evaluated. The Chinese hamster ovary (CHO) cell line is by far the most important mammalian cell line used for the production of therapeutic proteins.

Immortal hamster cells

All of today’s CHO cells that are stored in many hundreds of laboratories and production facilities around the world originate from a single female Chinese hamster that lived and died in 1957 in Theodore T. Puck’s laboratory at the University of Denver, Colorado. Puck’s extreme enthusiasm for the cultivation of CHO cells shows that the original hamster must have been a very unusual animal: “Cultured Chinese hamster lung, kidney, spleen and ovary cells divided rapidly; it was possible to keep the ovary cells in culture for more than ten months with no decrease in the cell division rate and no morphological changes.” (J. Exp. Med. 108, 945 ff., 1958). The CHO cells still had the same properties when Puck (whose actual name was Puckowitz) died in 2005 at the age of 89 following complications from a broken hip.

Theodore T. Puck was known worldwide as the “father of CHO cells” and he was also held in high regard for his groundbreaking work on the cytogenetics and culturing of human cells, without which the worldwide Human Genome Project would never have started. Nowadays, around 70 per cent of all recombinant biopharmaceutical ingredients used for the production of human medicines are produced in cells that originate from the immortalised primary culture of the ovary cells of Puck’s hamster.
The alleged immortality of CHO cells is not the only characteristic that has caused them to become popular objects of biopharmaceutical production. CHO cells can also be kept in suspension cultures; in contrast to cancer cells, they are genetically stable; they can be reproduced with expression vectors that contain the “gene of interest” (GOI); they can be transfected; and they remain stable during the process of selection, amplification, single-cell cloning and the characterisation of the clone. This process is very time- and labour-consuming and hence very expensive; it can take around five months to complete. The quality and the commercial success associated with the production of glycoproteins and monoclonal antibodies depend largely on the CHO clone used for this purpose and its level of productivity. Because this is an extremely competitive market, pharmaceutical companies take great pains to continuously optimise the individual steps of what is known as downstream processing. The details of optimising downstream processing steps is therefore one of the best kept secrets of companies involved in this kind of cell culture, whether this involves the characteristics of the expression system used for the GOI or for transfection, the composition of the culture medium, the feeding strategy (perfusion, for example) and the duration of the culture passages, pH value, temperature and cell concentration. All these aspects affect the cells’ viability and their productivity. Over the last twenty years, it has been possible to increase the number of cells that can be kept in culture 20-fold, to the extent that it is now possible to cultivate around 10 million protein-expressing cells per millilitre culture medium for three times as long as was previously possible.

The duration of the process that leads to the characterisation of a stable clone can be further reduced through the use of modern technologies. For example, quantitative PCR enables the rapid selection of cells and the image-based automated analysis of microtitre plates using Roche’s Cellavista system, for example, enables the identification and cloning of the most suitable cells contained in the amplified cell population.

The protein titre that can be achieved with bioreactors is a key cost factor. At present, it is possible to obtain therapeutic antibody titres of 3 – 5 grams per litre, and further research is focused on
increasing this value even further. The general trend is moving towards the use of smaller bioreactors and single-use plastic systems rather than stainless steel systems. Single-use plastic systems are more flexible and investment costs are lower: single-use systems can be constructed and put into operation very quickly; sterilisation and cleaning is inexpensive. Compared to standardised large-scale fully-automated stainless steel systems, personnel costs are higher because the semi-automated processes associated with the use of single-use systems require highly trained users and a larger number of manual steps.

Training of highly skilled people

Numerous companies have specialised in providing the necessary training required to handle cell cultures. Heidelberg-based PromoCell GmbH is one of these companies; the company offers further training courses for technical personnel and laboratory managers in the pharmaceutical industry as well as for post-doctoral students and lecturers at universities and research institutions. The company, which is also a leading producer of human cell cultures and cell culture media, offers laboratory courses, practical training and seminars that provide training in state-of-the-art cell culture technologies, including cell analysis, flow-through cytometry, viability and proliferation tests, microscopy, PCR and many other molecular biology and cell biology methods (PromoCell Academy). In addition to offering courses at the company's seminar centre in Heidelberg, the PromoCell Academy also offers "in house" training that is tailored to the requirements of the pharmaceutical and biotechnology industry. These seminars are being held in the clients' laboratories in order to give the participants the possibility to work with their employers' cell culture equipment.

The article is part of the following dossiers

Cell culture technology: it all started with frog nerve fibres