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REVIEW

Introduction to animal cell culture technology—past, present and future

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Although the first mammalian cell cultures were performed in the early 20th century to study cell physiology, it was not until the 50's that animal cell culture was performed at an industrial scale. The observation by Enders et al. (1949) that non-nervous tissue culture could be used to replicate and thus to produce polio virus paved the way to the large scale production of this urgently needed vaccine. This discovery led to the development of a production process for polio vaccine using primary monkey kidney cells and thus this vaccine became the first commercial product generated using mammalian cell cultures. Despite this break through, the use of primary monkey kidney cells is associated with many drawbacks, such as the relatively high risk of contamination with adventitious agents (contamination by various monkey viruses), shortage of donor animals, use of endangered animals as cell source, use of non-characterized or insufficiently characterized cell substrates for virus production, limited expansion and obligatorily adherent cell growth (Stones 1977; van Wezel et al. 1978; van Steenis et al. 1980; Beale 1981). In the sixties, human diploid fibroblast cells (WI-38 (Hayflick and Moorhead 1961), MRC-5 (Jacobs et al. 1970)) were established and Wiktor et al. (1964) could show that these cells could be used for the production of rabies virus for vaccine purposes. A larger production scale (use of 1 l bottles) was described and used for studying the immunogenicity of purified rabies vaccine in 1969 (Wiktor et al. 1969) (This production method was later used for the industrial production of an inactivated rabies vaccine (Nicolas et al. 1978)). In the same time (in the early sixties) BHK-21 (C13) cells were established and in 1964 the commercial production of inactivated FMD (Foot and Mouth Disease) vaccine was commenced by using a suspension process (based on the work by Capstick et al. (1962)). The Wellcome Foundation scaled this process up to a 2500 l scale (Pay et al. 1985; Radlett et al. 1985).

Two further important milestones were the Lake Placid Conference in 1978, at which the FDA accepted the use of continuous cell lines for the production of biologicals for human use (Petricciani 1995), leading to the commercial production of IFN (interferon) using the Namalwa cell line at a 8000 l scale (by the Wellcome Resesarch Laboratories) (Pullen et al. 1984), and the late seventies/early eighties, when the Institut Mérieux developed the Vero cells based production process and used it for the commercial production of inactivated polio vaccine. This process is a 1000 l microcarrier

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based production process (Montagnon et al. 1981, 1984a, b).

With the exception of the large scale production of IFN with Namalwa cells, there was no large scale production of other proteins before 1975, the year in which Köhler and Milstein (1975) published a paper describing monoclonal antibody technology. Ten years later, Celltech had already produced monoclonal antibodies at a 1000 l scale (Birch et al. 1987).

The eighties were marked by the advent of mammalian cells based expression systems of heterologous proteins: recombinant CHO technology, the development of the amplification and selection markers Dihydro-fdate-reductase (DHFR) and Glutamine-Synthetase (GS) (for Chinese hamster cells) and the development of recombinant baculovirus/insect cells technology. Then the early nineties were marked by the development of recombinant GS-NS0 technology (by Celltech) leading a few years later to the possibility of producing 1–2 g/l of monoclonal antibodies in a fed batch process (Bibila et al. 1994). Today we see antibody titers at an industrial scale of 5 g/l and more (Birch 2005).

The nineties have also seen the scale up of commercial reactor plants to 500 l for the production of monoclonal antibodies using a perfusion process (e.g. Centocor) and to 12,000 l (e.g. Genentech, Vacaville/CA—facility) and recently to 20,000 l (e.g. Lonza, Portsmouth/NH—facility) for the production of monoclonal antibodies and recombinant proteins.

Today, the biotechnology and pharmaceutical industrial sectors rely heavily on mammalian cell cultures as bio-production systems to manufacture various biological therapeutics including antibodies, interferons, hormones, erythropoietin, clotting factors, immunoadhesins, and vaccines. The market for monoclonal antibodies alone is expected to grow 30% a year and reached sales of over 6.5 billion US$ by 2004; and 16 further monoclonal antibody products are expected to reach the market in 2008, generating expected sales of further 3 billon US$ (Reichert and Pavlou 2004). The vast majority of these biological therapeutics are secreted glycoproteins obtained from mammalian cell lines such as Chinese Hamster Ovary (CHO), Human Embryonic Kidney (HEK-293), hybridoma and NS0 (murine myeloma) cells.

This brief historical review of key developments and achievements in animal cell technology seems to indicate that all is already developed and optimized and that no further developments will be needed. However, this is not the case. Although the largest production scale for batch or fed batch suspension cultures has already reached 20,000 l (and 2000 l for industrial microcarrier cultures (Tanner 2002)), not all scale-up issues, such as mixing, mixing times, mass transfer issues (O_2, CO_2), or shear effects, have been engineered totally satisfactorily. With respect to perfusion systems, the actual available retention devices used in industry have all very specific limitations and presently three different principles are used at the industrial scale: the spin filter system (e.g. at Centocor), the inclined settling device (e.g. at Chiron), and the ultra-sound retention device (BioSep from Applikon). The largest scale actually used in industry is a 2000 l stirred tank reactor, which is equipped with a spin filter (Tanner 2002). The review by Nienow in this issue deals with scale-up/scale-down and large scale stirred bioreactors in detail.

The standard reactor control is based on the control of only few physical parameters (pH, pO_2, pCO_2, temperature, redox). A very important parameter is the cell number per ml of reactor culture volume. Although several different principles, such as infrared cell density measurement (Merten et al. 1987), laser based cell counting/image analysis (e.g. Falkner and Gilles 1998; Joeris et al. 2002), or indirect follow up via metabolic analysis, have been developed and evaluated, the radio-frequency impedance measurements system seems to be the most interesting. This superiority is because in the latter case, only the living cell number is measured, whereas for the other methods either the total cell number (Merten et al. 1987) or the living cell number via sophisticated image analysis (Falkner and Gilles 1998; Joeris et al. 2002) or an estimation of the metabolically active biomass are provided. The radio-frequency impedance measurement technique and its industrial applications are reviewed by Carvell and Dowd (in this issue).

Although R&D in the frame of reactor and vaccine development is still ongoing, the main R&D activities in animal cell technology today deal with global optimization, cell analysis, and new approaches to providing more patient specific/destined medicine.

For the last 10–20 years, the tendency in industrial animal cell technology has been towards the use of serum-free or protein-free media with the very important fact that these media should be ''animal-free''—for biological safety reasons, of course. The development and optimization of serum or protein-free media is a rather complex undertaking, and serum, serum–proteins as well as single amino acids can efficiently be replaced by recombinant proteins (review by Keenan et al. this issue), peptides from plant protein hydrolysates and synthetic oligopeptides (Franek et al. 2000, 2003; Franek and Katinger 2002; Franek and Fussenegger 2005) and/or protein hydrolysates (Keenan et al. this issue). Whereas the use of serum-free media is largely established for the production of recombinant proteins, the classical viral vaccine production processes still make use of serum-containing media at least during some phases of the process (Merten 2002) although such processes can be efficiently performed under serum or protein-free conditions, such as for the production of polio virus by Vero cells grown under serum-free conditions (Merten et al. 1997). New developments in the vaccine field clearly make use of serum-free media (Merten et al. 1996; Brands et al. 1999; Kistner et al. 1999; Makoschey et al. 2002; Gilbert et al. 2005).

One of the most important activities is the use of general cell and metabolic engineering. This approach aims to improve certain crucial cellular functions, such as metabolic pathways and the overall cellular physiology; the cellular behaviour against environmental stress conditions; or the expression of lacking or the increased expression of insufficient levels of ER or Golgi based enzymes, necessary for co- and post-translational modifications when they are lacking or their increased expression if their amount is insufficient.

With respect to the optimization of the cell's physiology, the reader is refered to some interesting papers. Some examples are the use of the GS as a selection and amplification system and also as an optimization approach for the cellular metabolism to get rid for the need for glutamine in the culture medium (Sanders et al. 1987; Bebbington et al. 1992; Brown et al. 1992); or the overexpression of cytosolique pyruvate carboxylase in continuous cell lines for improving the connection between glycolysis and the Krebs-cycle (Irani et al. 1999: BHK21; Elias et al. 2003: HEK293); or the overexpression of anti-sense LDH-A and of cytoplasmic glycerol-3-phosphate dehydrogenase in CHO cells for increasing the oxidative phosphorylation, for decreasing cellular respiration and thus reducing sensitivity to reactive oxygen species and overall, reducing apoptosis (Jeong et al. 2004). Cellular behaviour against environmental stress conditions is often characterized by an induction of apoptosis, thus leading to a precious loss of viable biomass and the stopping of a productive culture. The review by Arden and Betenbaugh (this issue) deals with the general problem of apoptosis in animal cell culture and with various means (culture and metabolic engineering) for remedying it.

One important objective of today's R&D in animal cell technology is the production of consistently glycosylated proteins (or, in general, of proteins which have consistently experienced the correct co- and post-translational modifications). The paper by Butler in this issue deals with the influences of culture media and cell line characteristics on the glycosylation of recombinants proteins as well as attempts using metabolic engineering to manipulate and control the metabolism of cells for producing recombinant proteins with a human like glycosylation profile. Further cellular optimizations concern all other co- and post-translational modifications which a given protein can experience before secretion, such as multimering, cleaving, formation of -S-S- links, phosphorylation, sulphatation, etc. For more information, the reader is referred to papers dealing with the overexpression of different chaperones, such as BIP (Dorner et al. 1993; Hsu and Betenbaugh 1997), PDI (Davis et al. 2000), calnexin-reticulin (Chung et al. 2004), or endo-proteases necessary for the cleavage of pro-peptides (Preininger et al. 1999).

However, the optimization of a producer cell lines does not only depend on the right choice of the producer cell line and the culture conditions, but also on the genetic constructions of the expression plasmids, the use of cassette exchange approaches to get to well characterized mother cell lines that do not loose their overall behaviour when different genes have to be expressed (reviewed by Oumard et al. in this issue) and on the use of inducible expression systems for various purposes (review by May et al. this issue).

Rather recent developments in optimization technologies make use of mini-reactors, which allow the rapid optimization of culture conditions relevant to an industrial setting by using for instance statistical experimental plans (Stäheli 1987; Gaertner and Dhurjati 1993a,b; Kallel et al. 2002; Deshpande et al. 2004). In this category of culture systems, reactor scales ranging from some 100 mls (the Infors AG Sixfors system and the DASGIP system, both using standard industry sensors for process control) over some milliliters (48 stirred-tank reactors arranged in a bioreaction block (Puskeiler et al. 2005; Weuster-Botz et al. 2005) or a 12 mini stirred-tank bioreactor system equipped with disposable DO and pH optical sensing patches (Ge et al. 2006)) down to several microliters (e.g. development of high-throughput bioprocessing devices based on the use of standard well plates (Girard et al. 2001)) are included. However, in order to complete a high-throughput optimization, the mini-reactor approach has to be coupled to genomic and proteomic approaches, being very powerful means for analyzing the cellular behaviour under (or cellular reactions to) different culture conditions. The general overview by Wlaschin et al. (this issue) deals with the aspects of a profound cellular analysis which can be used as rational base for the general optimization of producer cell lines and culture conditions, as has already been done by Allison et al. (2005) for the development and optimization of serum-free media for human cell lines.

The future of animal cell technology will see an enlargement of its applications towards novel domains, such as, the use of viral vectors for gene therapy, which is in some way an enlargement of vaccine technology. However, it needs many additional developments, first in the domain of molecular biology and molecular virology for developing the viral vectors (rendering viruses a-pathogen and apt for gene transfer) and second in the field of animal cell technology with respect to the development of helper and producer cell lines or the development of 'advanced' means for vector production (e.g. the use of the Sf9/baculovirus system for the production of AAV (Urabe et al. 2002)). An update on production issues of viral vectors for cell and gene therapy purposes is given by Warnock et al. (this issue).

Classical animal cell technology has been concerned with the production of viruses, viral vectors, and recombinant proteins for different purposes. However, animal and human cells can also be used for patient destined therapies (artificial organs, tissue engineering, transplantation of cells). This is a rather new field and many human diseases (acquired and inherited) will be treated by these kinds of therapies in the future. On one hand, these therapies are based on gene therapy (ex vivo–in vivo) by using viral vectors (see above) and on the other hand by the development of artificial organs (use of patient destined small scale reactor systems, employable in a clinical setting) and tissue repair/tissue engineering, based on the use of stem cell technology have to be mentioned here. In the context of artificial organs, one of the most important potential applications is the transient replacement of a patient's damaged liver by an artificial one, thus bridging the time gap between liver failure and the availability of one from a matching donor or the regeneration of the patient liver. Different culture approaches are reviewed by Diekmann et al. (this issue). In the future, tissue repair can be achieved by tissue replacement based on the use of stem cells. For this purpose, adult as well as human embryonic stem cells can be used. For a general introduction to this domain, the reader is referred to the special issue of Cytotechnology on 'Stem Cell Biology and Clinical Applications' (vol. 41, nos. 2–3, edited by S. Eridani and L. Cova). In addition, an overview on a very recent and highly publicized domain with very high expectations (economic as well as medical)—the use and amplification of human embryonic stem cells (HESC)—optimization of culture con-

ditions, etc., was prepared by Oh and Choo (this issue).

Finally, in assembling this special issue, the aim was to present the most important advances and future perspectives in the domain of animal cell technology via invited reviews/overviews and the provision of key references for going deeper into the respective subject areas.

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