Large-Scale Mammalian Cell Culture Technology

edited by

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Series Introduction

Bioprocess technology encompasses all of the basic and applied sciences as well as the engineering required to fully exploit living systems and bring their products to the marketplace. The technology that develops is eventually expressed in various methodologies and types of equipment and instruments built up along a bioprocess stream. Typically in commercial production, the stream begins at the bioreactor, which can be a classical fermentor, a cell culture perfusion system, or an enzyme bioreactor. Then comes separation of the product from the living systems and/or their components followed by an appropriate number of purification steps. The stream ends with bioproduct finishing, formulation, and packaging. A given bioprocess stream may have some tributaries or outlets and may be overlaid with a variety of monitoring devices and control systems. As with any stream, it will both shape and be shaped with time. Documenting the evolutionary shaping of bioprocess technology is the purpose of this series.

Now that several products from recombinant DNA and cell fusion techniques are on the market, the new era of bioprocess technology is well established and validated. Books of this series represent developments in various segments of bioprocessing that have paralleled progress in the life sciences. For obvious proprietary reasons, some developments in industry, although validated, may be published only later, if at all. Therefore, our continuing series will follow the growth of this field as it is available from both academia and industry.

W. Courtney McGregor

Preface

Large-scale cell culture is a hybrid technology that combines aspects of many different disciplines, including cell biology, biochemistry, fermentation, virology and microbiology, and protein chemistry. This technology has been employed historically to prepare virus vaccines, but more recently to produce protein pharmaceuticals. The need for these products to be safe, effective, and economical has provided the impetus for developmental efforts. These efforts have led to improvements in the technology and are occurring regularly. This growth, which has been especially noticeable over the past 7 years, has prompted the present work to be written.

This book critically reviews the current state of the field. The aim of the book is to offer its readers a view of what is happening and how the different disciplines interact to produce safe products. To understand that process it is necessary to examine each specialty and to describe the benefits, shortcomings, and interactions of each.

From the genesis of this work, the chapter authors and I felt this book should be of value in stimulating the current generation of graduate students to enter the field of cell culture technology. We also wish to encourage professionals of related fields to diversify into cell culture technology. At this time, more trained workers are needed to satisfy the current industrial demand and to increase the rate at which new discoveries can be made.

Accordingly, we have prepared a manuscript that strives to be comprehensive, readable, and critical. The chapters include basic theory as well as current applications and problems. Wherever possible, interrelationships of diverse concepts are illustrated. We hope that this interdisciplinary approach to cell culture methodology will stimulate readers to start or continue a career in this complex, but rewarding, area of technology.

Anthony S. Lubiniecki

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Large-Scale Mammalian Cell Culture: A Perspective

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1. INTRODUCTION

While the large-scale culture of mammalian cells may seem a comparatively recent development, both human (1) and hamster cells (2,3) had already been grown in 1000-liter culture tanks some 20 years ago. Work on such a scale was at first carried out in only a very few institutions whose special needs made it worthwhile to tackle the initially formidable technological and other problems. One such need was to produce foot-and-mouth disease (FMD) virus vaccines on a commercial scale for veterinary use; another was to make large amounts of human interferon (IFN) for clinical evaluation. Elsewhere, large-scale cell culture was generally thought to be a difficult, clumsy, and uneconomical way of manufacturing, with the further disadvantage that its products might in the end prove to be unacceptable for use in terms of safety.

Concerns about safety stemmed from studies in the 1950s and 1960s with cultures in magnetically stirred "spinner" flasks. These showed that the cells which grew readily in suspension culture and which could be subcultured without apparent limit were all "transformed" and indeed could be distinguished by these characteristics from "normal" cells grown directly from human or animal tissues, and diploid fibroblasts. Most transformed cells were derived from

tumors, were aneuploid with at least some chromosomal abnormalities, and when injected into neonatal or immunosuppressed animals led to the formation of tumors. Since little was known at that time about the mechanisms responsible for the transformed state, the idea of using such cells as a source of medicinal products met with little favor: it was feared that the products might be contaminated with some virus or oncogenic factor derived from the cells with potentially disastrous consequences for the recipients.

Today, attitudes have changed as the result of developments in the scale and reliability of mammalian cell culture and the proven safety and efficacy of proteins derived from them. In many countries there are now regulations governing pharmaceutical manufacture from suspension cell cultures and a number of products made in this way have been licensed for use. Indeed, mammalian cell cultures are now the preferred source of a number of important proteins for use in human and animal medicine, especially those which are relatively large, complex, or glycosylated. This chapter will trace the developments which have influenced present views, with special reference to the important precedents which FMD virus vaccines and interferon have provided.

II. PRODUCTION OF FOOT-AND-MOUTH DISEASE VIRUS VACCINE FROM BHK 21 CELLS

Foot-and-mouth disease (FMD) is enzootic in cattle, sheep, goats, and pigs in many parts of the world and, unless controlled, has major economic consequences through its effects on animal productivity and international trade. In 1947, Frenkel (4) showed that FMD virus could be grown in surviving fragments of fresh bovine tongue epithelium incubated in a suitable medium. Today such cultures, handled in tanks on an industrial scale, still provide virus which is used to make vaccines. However, this system has the disadvantages common to all primary tissue culture systems and cannot provide enough vaccine to satisfy the worldwide demand.

In 1962, Mowat and Chapman (5), working at the U.K. government Animal Virus Research Institute (AVRI) at Pirbright, succeeded in growing FMD viruses in the anchorage-dependent cell line, BHK21, clone 13 (6). In further work, Capstick and coworkers at the AVRI adapted BHK cells to grow and replicate FMD viruses while in suspension culture (7). Further studies by these workers at the AVRI and subsequently in the adjoining Wellcome FMD laboratories defined the environmental conditions needed for optimal cell growth and production of viruses, and showed how the process could be scaled up

for industrial use. These various studies identified both the similarities and the differences between mammalian cell culture and the systems already established for microbial culture; they will be described in some detail since many of the developments which turned the initial laboratory experimental system into a robust industrial process have proved to have general application in large-scale mammalian cell culture work.

Initial experiments at AVRI in 200- and 800-ml glass vessels (8,9) were soon scaled up into stainless steel vessels of 30- and 100-liter capacity, based on those available for the submerged culture of bacteria (10–13). They were made of an austenitic chromium–nickel alloy, with internal welds ground smooth and the internal finish a dull polish. The vessel ends were dished and the lid was attached to the body with a full-faced flanged gasket. To reduce the number of connections into the tank and consequent sterilization problems, pressure relief safety valves were not fitted; instead, the vessels were designed for a working pressure of 60 psi, and air, steam, and other services were supplied at maximum pressures well below this limit. Valves in contact with the culture media were of the diaphragm type or were stainless steel ball-plug valves in which leakage via the spindle is prevented by a self-adjusting polytetrafluoroethylene diaphragm seal (14).

The cells were stirred with a single-vaned disk impeller mounted on a stirrer shaft which passed through a double mechanical seal. Culture temperature, pH, and stirrer speed were automatically controlled (3,11,13). Temperature was found not to be critical over the range 33–37°C. The bicarbonate-CO_2 buffering system proved inadequate when the cell number exceeded about a million cells/ml and the pH fell below 7.0. Therefore, to improve pH control, air was automatically injected below the impeller when the pH fell below the desired value, and CO_2 similarly when the pH was too high (11,12). This method controlled pH to within ±0.05 pH unit. At a temperature of 35°C and a pH of 7.4, a fivefold increase in BHK cells to about 2.5×10^6/ml was consistently obtained within 48 hr (2,3,13,14). The cells grew best at a pO_2 level of 80 mm Hg, but in-place oxygen electrodes proved unstable and subject to fouling during prolonged culture (15,16). Since Tengerdy (17) had shown that the redox potential difference between aerated and deoxygenated culture medium was linearly proportional to the logarithm of the dissolved oxygen concentration, redox electrodes were installed: these controlled the automatic addition of sparge air whenever the redox potential fell below a desired value, approximately equivalent to a pO_2 level of 40–80 mm Hg. With this indirect method, the rate of growth and cell densities matched those resulting when

direct aeration measurements were used for control (Telling, unpublished observations).

Master cell banks were fully characterized according to U.S. federal requirements (18) and the cells were stored frozen at −65°C or in liquid nitrogen. All cells used in production were revived from such a bank.

An inexpensive medium based on Eagle's formula, supplemented with 5% adult bovine serum and tryptose phosphate broth, was developed which gave good productivity in the tank cultures. The complete medium was sterilized by filtration through asbestos-cellulose sheets (19) held in filter holders permanently connected to the culture tanks. These and the connecting pipework were sterilized by steam injection; as a precaution, the pressure within the system was kept positive throughout the cycle of sterilization, cooling, and filtration.

The integrity of the vessels was improved by the development of magnetic stirring (20):. a driven magnet immediately adjacent to the outside of the upper dished end of the tank induces rotation of a follower magnet mounted immediately opposite within the vessel on an axle shaft bearing the impeller. This elimination of a continuous drive shaft with its attendant mechanical seals reduced the chances of accidental microbial contamination. For the same reason, hard-piped steam-sterilizable sample and addition points were added. These changes in plant design together with attention to details in plant operation improved the long-term maintenance of sterility with the closed system of tanks and pipes, and enabled the use of a "solera" system of culture which has proved indispensable for larger scale culture work. In this system, cells are grown for months at a time in continuous culture in a particular tank; cells are drawn off as required to feed the virus production vessels, leaving a volume of cell suspension in the solera tank which is diluted with fresh growth medium, so that fresh cycles of cell growth take place. This major difference from the practices used in microbial fermentation reflects the very much slower rate of growth of animal cells; several weeks may be needed from the time cells are revived from the master cell bank until there are sufficient cells to seed the largest vessels.

Experimental work confirmed that reproducible growth of the BHK21 cells and production of FMD viruses could be obtained and that successive batch cultures could be carried out in the same tank over extended periods of time without problems from microbial contamination.

FMD virus strains grown in the system described were the source of inactivated vaccines which were shown to be potent and to protect animals of the target species against challenge infection

(20,21). As already pointed out, there were initial concerns that a vaccine of such an origin might prove to be tumorigenic. BHK cells contain C-type virus particles; they also become aneuploid and have an increasing oncogenic potential in the species of origin — the hamster — with successive passages in suspension culture (22, 23). However, a product for veterinary use enables direct safety tests to be carried out in the target species; accordingly, high concentrations of cells and cell extracts containing nucleic acids were injected into animals of various species, which were observed for periods of up to 2 or 3 years (individual animals and groups have been followed for periods up to 16 years; Garland, unpublished). All these studies confirmed that tumors only developed in one species, the hamster, and then only if intact cells were injected (22,23); the vaccine production process ensures that all cells are destroyed or removed during the course of manufacture.

The technology thus developed on the pilot plant scale has been used since 1967 for the design and operation of the industrial scale FMD virus vaccine production plants of the Wellcome (now Cooper Animal Health) group, located in eight countries in Europe, Africa, and South America. These now utilize vessels of up to 5000-liter capacity to produce the particular FMD virus serotypes of local importance. By 1976 more than 1 million liters of vaccine had been issued annually from these plants, and by 1983 approximately 2 million liters of vaccine, equivalent to 350 million doses of monovalent vaccine (20,24). The same basic technology has been used by Wellcome to make inactivated rabies virus vaccine for veterinary use from BHK 21 cells (25,26).

III. MASS CULTURE OF LYMPHOBLASTOID CELLS AT THE ROSWELL PARK MEMORIAL INSTITUTE

While FMD virus vaccines were being developed in the United Kingdom, a pioneer study was under way at the Roswell Park Memorial Institute in upper New York State. George Moore and his colleagues constructed a plant for growing human lymphoblastoid cells in suspension in a 1000-liter culture tank (1), at the time the largest scale used for mammalian cells. Their study showed that work at this scale was feasible and led to the development of a number of media, particularly RPMI 1640, which are still used for the culture of lymphoblastoid cells. However, no uses could then be found for the cells which were produced in large quantities; financial support ceased and the plant was abandoned.

This project was ahead of its time, and with hindsight it seems very likely that the harvests from some of these lymphoblastoid cell cultures contained various lymphokines in quantities sufficient to permit their isolation and purification, had this been realized.

IV. DEVELOPMENT OF LYMPHOBLASTOID INTERFERON AT WELLCOME

The clinical evaluation of the interferons (IFN) was greatly impeded by a supply shortage for more than two decades after the original discovery in 1957 (27). Leukocyte IFN preparations were made from human white blood cells obtained from transfusion blood (28), and these enabled clinical studies to start in the early 1970s, but only relatively small amounts could be made in this way.

At the Wellcome Research Laboratories in Beckenham, studies with IFN started in 1959. In 1974, Finter and colleagues decided to explore alternatives to human blood cells as a source of IFN in order to make substantially larger amounts available for clinical trials. By then, Wellcome staff already had considerable experience with the culture of BHK cells in tanks, and so it was logical to see if IFN could be made in a similar system. After many human cell lines had been screened (29), the Namalwa line of lymphoblastoid cells was selected for further study, as these cells grew well and formed large amounts of IFN. The program led to the large-scale production of a highly purified human lymphoblastoid IFN preparation, which is now the commercial product, Wellferon. After preliminary scale-up work in a 50-liter tank, a 1000-liter pilot plant was commissioned early in 1978; this was followed by production from plants based on 2000-liter vessels in 1980, and on multiple vessels of 8000- or 10,000-liter capacity from 1983 onward.

Experience soon showed that while the equipment and technology needed to make FMD vaccines and IFN are basically similar, the latter process is technically much more demanding, reflecting the more fastidious cell, the more complex production system, and the more stringent requirements for a product intended for human use.

Namalwa cells grow relatively slowly, with a doubling time of about 35 hr, and are therefore cultured in a continuous solera system. In larger plants, solera vessels in a series of increasing size generate the cells needed for IFN production and provide a source for rapid reseeding if one of the series is lost for whatever reason. IFN is made in a batch process in which cells are transferred to a production tank, treated with butyrate, and subsequently induced by the addition of Sendai virus (30). The production vessels are sterilized between each batch of cells, but the solera tanks are maintained in continuous culture for as long as possible. With attention to detail in design and operation, the average duration of the solera cultures has been progressively extended from a few weeks on the pilot plant to 10 months or more on the present plants, with great benefits to the efficiency and economy of production. Factors