MAMMALIAN CELL CULTURE

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Keywords: Biopharmaceuticals, bioreactors, cells, hybridomas, mammalian, media, metabolism, physiology, protein, tissue culture

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Summary

Mammalian cell culture has a bright yet changing future. Many challenges remain and new areas will open. The cell culture technologist will continue to work with cell biologists, biomaterials scientists, clinicians and regulatory authorities to produce

efficacious and safe products to patients. In all areas, the development and application of protein-free media remains a priority to assist purification and ensure the use of biologically safe raw materials. Process intensification, particularly in generating gene therapy viral vectors and in recombinant protein and antibody production remains an important issue to generate sufficient material and lower production costs. In the field of recombinant protein production cell culture-based systems face competition from developments in emerging technologies such as production in transgenic animals. Virus production, whether for vaccines or for gene therapy, will however, inevitably be produced in some type of mammalian cell.

There are many exciting areas which have not been reviewed in this chapter. One in particular is the culture of mammalian cells for toxicity testing or drug screening. This technology will increase in importance because of the vast array of natural and combinatorial products that require screening and the increased pressure to reduce animal testing.

In summary, this review has focussed primarily on the biotechnological aspects of mammalian cell culture. In the twentieth century, mammalian cell culture developed from its infancy, to providing a vehicle for viral vaccine production and most recently to produce monoclonal antibodies and other recombinant proteins. As we enter the new century, many more biopharmaceuticals, produced by mammalian cell culture will become available. Cell culture technology will play an important role in the emerging fields of gene therapy and tissue engineering.

1. Introduction

Mammals are comprised of a large variety of cell types with specialized functions including:

- Providing structure, scaffolding and support
- Signaling and sensing
- Digestion and absorption of foods and water
- The transfer of molecular oxygen
- Immunity
- Motor function
- Reproduction

The vast array of cells that support these (and many other) functions arise from very few cell types by a process collectively known as differentiation [see *Cell Theory, Properties of Cells and their Diversity*]. The elegance with which these cells develop, grow, regenerate and indeed die has been partly elucidated by scientist studying the cells *in vitro* (literally, "in glass" or outside the body). As our ability to grow and support cells in this way has developed, so has our knowledge of cell function and physiology [see *Eukaryote Cell Biology*].

Humankind has also utilized *in vitro* cell culture technology to produce a range of viral vaccines and protein biopharmaceuticals. More recently, cell culture technology has

been applied to the culturing of mammalian cells for re-transplantation and increasingly, cultured cells are used in toxicity screening.

The purpose of this chapter is to introduce the reader to the science and technology of *in vitro* mammalian cell culture and to provide an appreciation of the impact that cell culture technology has on the health and well-being of mankind. Being a large topic area, this review focuses on the biotechnology of cell culture: use of mammalian cells to produce products or the culture of cells as products in their own right.

2. A Brief History of Mammalian Cell Culture

The period from 1880 to the early 1900s saw the first development techniques to study the behaviour of cells *in vitro*. Although Harrison is normally accredited with the development of cell culture as a scientific tool, he described his own work as an extension of Wilhelm Roux (1885). Both these scientists were interested in studying specific forms of cellular differentiation during embryo development. Roux however was not concerned with the multiplication of cells in culture. Harrison's "hanging drop" experiment enabled observation of the growth of nerve cells from the original explant and was able, with care, to maintain sterile growth for up to four weeks, which stimulated an expansion of interest in the science of *in vitro* cell growth and development.
Burrows established mammalian cell culture using chick embryos as the source of cells grown in the presence of plasma clots using Harrison's method. A significant development made by his group was the demonstration of the principle of media exchange and sub-culture. Burrows and other workers demonstrated growth of epithelial cells, connective tissue and a variety of tumour cells. Continuous passage of cells demonstrated by Ebeling and others led to the conclusion that somatic cells could survive indefinitely *in vitro* if media was replaced and conditions were appropriate.

The first permanent cell line was developed by Earle in 1943 from subcutaneous mouse tissue. Cell cultures were propagated continuously (designated strain L) and were shown to be morphologically quite different from the original tissue. Thus it was shown that "transformed" cell lines could be developed. The first human "transformed" cell line was the HeLa cell, derived from a cervical carcinoma.

Other techniques that came to be important for both small-scale and large-scale cell culture were developed during the 1950s and 1960s. These included the use of trypsin to permit sub-culture of attached cells from one flask to another, developments in cell culture vessels and bioreactors, methods of cell cryopreservation and developments in cell culture media formulations.

One of the first significant applications of cell culture arose from the observation that polio virus could be cultured in simian and human kidney cells as opposed to nerve tissue. [see *Immunobiotechnology*].When treated with formalin, the resulting preparation was immunogenic and provided prophylaxis for polio infection (it became known as the Salk vaccine). Live attenuated vaccines for polio were soon developed, also produced in kidney cell lines.

Another development during this period was the development of cell culture methods for the production of rabies vaccines. Such vaccines were originally produced from inactivated infected tissue (another of Pasteur's contributions to science), but were not always safe and contained a whole range of non-specific antigens. The development of diploid cell lines such as MRC-5 and WI-38 (human origin) and Vero (simian) permitted safer, cheaper and more efficacious preparations of rabies virus for vaccination. During the 1960s, mammalian cell culture methods were developed to produce vaccines for other diseases such as measles, mumps and rubella in humans and foot-and-mouth disease for veterinary application.

The development of technologies to economically express proteins from mammalian cells began in the 1970s and 1980s. The production of interferon in a human lymphoblastoid line was established and large-scale production achieved. Monoclonal antibody technology facilitated the development of continuous cell lines that could secrete antibodies of defined specificity. This has had an enormous impact on the ability to design and produce useful diagnostic and therapeutic antibodies. The first monoclonal antibody licensed for therapeutic use was the murine antibody, OKT3. Through developments in recombinant DNA technology, phage display and molecular biology, the ability to produce fully human antibodies, antibody fragments and bi-specific antibodies of commercial potential is being achieved. Other recombinant proteins produced in mammalian cell lines such as Chinese Hamster Ovary (CHO) cells include tPa (an anti-thrombolytic agent) and erythropoietin (a glycoprotein that stimulates red blood cell synthesis *in vivo*).

Cell culture products have a distinguished place in their contribution to developments in healthcare in the twentieth century. With the almost exponential increase in cell culture derived products currently in clinical trial, our world can expect an ever-increasing impact from biopharmaceuticals produced by mammalian cell culture. New developments in tissue engineering and gene therapy [see *Gene Therapy*] make it an exciting time for cell culture technology.

3. Primary and Continuous Cultures

3.1. Primary Cultures

There are *in vitro* cultures of cells obtained directly from the organ of interest. Cultures are considered primary until the first sub-culture (where cells are taken into a new bioreactor or flask with fresh medium, often at a lower concentration). Primary cell cultures are used in areas of research such as cellular metabolism and physiology, cell morphology and genetics.

Cell cultures may be initiated from normal, embryonic or malignant tissue by aseptic collection from the animal host. In some cases, physical or enzymatic methods are used to disrupt the cells to prepare suspensions of single cells. Commonly, a variety of different cells are obtained from a single blood or tissue sample. Thus, primary cultures are often "mixed" (different cell types present) and commonly the *in vitro* culture conditions are such that certain cell types (e.g., fibroblastic) dominate over the other cell types over time.

3.2. Continuous Cell Lines

Following serial sub-culture of primary cells, cells that continue to grow become cell lines. These are sub-populations of the original primary tissue that are capable of continual growth, provided they are supplied with nutrients via periodic media change. These cell lines are often very useful because they can be stored in liquid nitrogen and revised for later use, without having to obtain another tissue sample. Cell lines may be finite (will continue to grow only for a limited number of population doublings) or continuous, where cells will proliferate indefinitely, provided they are serially passaged into fresh media. For cell lines to become continuous, an "immortalisation" event must occur. This may occur naturally in culture (by somatic mutation), by viral transformation, by other induced mutation or by hybridization (fusion of the host cell with an immortal cell line).

Continuous cell lines may be utilised for the production of biopharmaceuticals, vaccines, gene therapy vectors or as fusion partners with other cell types. A host of cell lines have found use in a variety of research and industry. Most of these cell lines are "archived" in cell "banks". The predominant organizations for these are the American Type Culture Collection (ATCC) in the USA and the European Collection of Animal Cell Cultures (ECACC). These organizations are important because they allow world access to a variety of cell hosts which have been tested and their histories documented. This standardization assists experimental reproducibility and regulatory approval procedures.

A number of continuous cell lines have found use in the biopharmaceutical industry. Some of these are summarised in Table 1. The characteristics of three of these cell lines (VERO, CHO and myeloma cells) are discussed in more detail in section 3.4.

Cell Line	**Origin**	**Features**	**Use**
MDCK	Dog/Kidney	Epithelial like	Veterinary Vaccine
CHO-K1	Hamster/Ovary	"	Recombinant Proteins
VERO	Green Monkey/Kidney	Fibroblast	Viral vaccines (polio)
BHK-21	Hamster/Kidney	Fibroblast	Recombinant proteins Veterinary vaccines (Clone B)
MRC-S	Human/Lung	Fibroblast, Finite	Human vaccine
W1-38	Human Embryonic/Lung	Fibroblast, Finite	Human vaccine
Namawala	Human/Lymphoid (spleen)	Suspension	Interferon production
Mouse Myeloma	Spleen Sp2/0 and NS1	Suspension	Fusion to make hybridomas

Table 1. Cell lines used in biopharmaceutical production

3.3. Organ Culture

Finally, organ culture may be performed, in which part or all of an organ excised from an animal may be perfused with media and sustained under aseptic conditions. Though much knowledge has been derived from studying individual cell types in culture, in most cases, cells in organs respond to other cell types in the same (or different) organs. To observe this interaction, whole organ culture may need to be performed to understand the cell behaviour more completely [see *Tissue Engineering : Advances in Organ Replacement*].

3.4. Some Useful Cell Lines

3.4.1. CHO Cells

The Chinese Hamster Ovary Cell has been shown to be a popular, robust cell line for the production of recombinant proteins [see *Industrial Recombinant Protein Production*]. A number of therapeutic biopharmaceuticals are produced by recombinant CHO cells, including erythropoietin and tissue plasminogen activator. It has proven to be adaptable to serum-free and suspension cell growth. The use of the cell line became popular because of the wide availability of a mutant line deficient in the enzyme dihydrofolate reductase (DHFR). Expression vectors containing a DHFR gene can be used to isolate recombinant clones and amplify expression levels. Many other expression systems have subsequently been developed for CHO cell recombinant protein expression.

3.4.2. VERO Cells

VERO cell lines are continuous cell lines derived from African Green Monkeys (*Cercophithecus aethiops*) originally described by Yasamura in 1963. The name VERO is derived from Verde (French for green) and RenO (French for kidney). It was obtained by the ATCC at passage 93 from Chiba University Japan and submitted at passage 113. The VERO cell line has an epithelial morphology, non-tumorigenic (below passage 191), highly anchorage dependent and susceptible to infection by a wide range of viruses, including the reovirus family. These properties have led to small and large-scale culturing of VERO cells as cell substrates for the production of viral vaccines including vaccines for poliomyelitis and rabies.

3.4.3. Sp2/0 Myelomas

The Sp2/O-Ag14 cell line was isolated in the laboratory of Georges Kohler and reported in 1978. It has been one of the most widely used myeloma fusion partners for the production of antibody secreting hybridomas. Its specific advantage was that as a parent line, it produced no immunoglobulin chains of its own. Therefore all antibody genes in the resulting hybridomas would arise from the lymphocyte fusion partners. The line is also sensitive to a mixture of hypoxanthine (H), azoguanine and thymidine (T), permitting selection of hybrids from unfused myelomas. Furthermore, it is amenable to genetic manipulation and can therefore be used to produce recombinant antibodies or other proteins.

4. Methods in Mammalian Cell Culture

Different cell types require different conditions for cell maintenance, proliferation and product formation. Although methods applied in different laboratories, working with different cell types may vary, some features of mammalian cell culture are generically important features of mammalian cell culture. In this section, the focus will be on general methods for small and large-scale cell culture, rather than on specific assay methods.

Common features of *in vitro* mammalian cell growth include:

- Slow growth rates of cells
- Complex metabolism and possibly complex media requirements
- Possible requirement for surface attachment
- Relative fragility of some cells
- Genetic and phenotypic instability of some cell types

Cell culture systems need to accommodate these features, whether the cells are being cultured in the laboratory at volumes of 100 ml or at a pharmaceutical factory, where mammalian cells are grown to scales of 10 000 L.

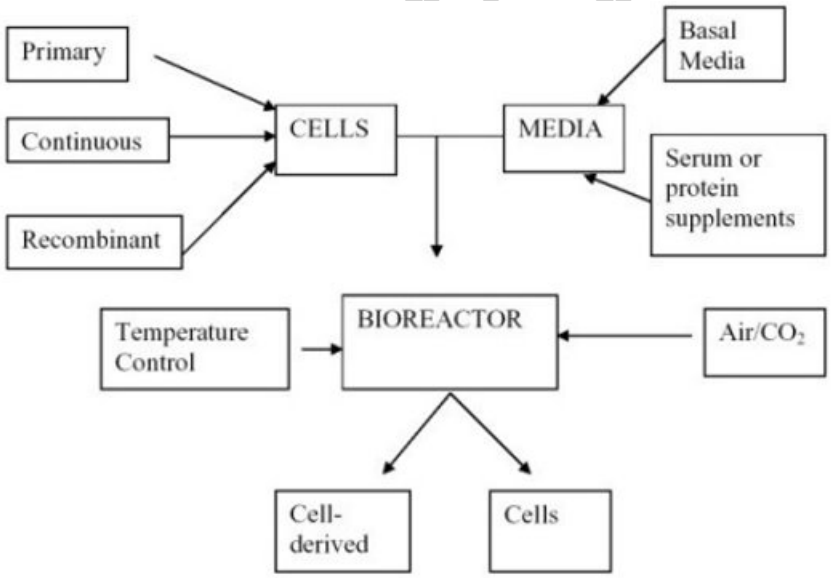

Figure 1: Inputs and outputs in mammalian cell culture systems

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Biographical Sketch

C. P. Marquis joined the Department of Biotechnology at the University of New South Wales at the beginning of 1994 after completing his Ph.D in the area of productivity improvements in the culture of murine hybridomas and human lymphoblastoid cell lines. His current research interests include prokaryotic and eukaryotic recombinant antibody expression, the application of green fluorescent protein in bioprocess research, the production of proteases by halophilic bacteria and the identification of bioactive compounds in Scleractinian corals.

He has worked with a number of groups outside the University including the Red Cross Blood Transfusion Service in Sydney and the Australian Institute of Marine Science (AIMS) in Queensland. He also works with a number of companies on industry related projects, including antibody and recombinant protein production and purification. In the second half of 1997, he undertook a 6-month study leave at Merck & Co., Inc at Rahway, NJ. During this period, he was associated with a Bioprocess Development group in the area of viral vaccines.

He teaches in a number of subjects, but is principally involved with teaching related to bioprocess engineering. More recently he has been involved in the development of distance education modules in the areas of food biotechnology, biopharmaceuticals and in brewing science. He has also taught in workshops in Indonesia and Thailand.