

# Chapter 1

## Mammalian Cell Culture Technology: An Emerging Field

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**Abstract** Mammalian cell culture technology has become a major field in modern biotechnology, especially in the area of human health and fascinating developments achieved in the past decades are impressive examples of an interdisciplinary interplay between medicine, biology and engineering. Among the classical products from cells we find viral vaccines, monoclonal antibodies, and interferons, as well as recombinant therapeutic proteins. Tissue engineering or gene therapy opens up challenging new areas. Bioreactors from small- (ml range up to 10L) to large-scale (up to 20m<sup>3</sup>) have been developed over the past 50 years for mammalian cell culture-based applications. In this chapter we give a definition of mammalian cells and a brief outline of the historical development of mammalian cell culture technology. Fields of application and products from mammalian cells, as well as future prospects, are discussed.

### 1.1 Definition and History

Mammalian cell culture technology has become a major field in modern biotechnology, especially in the area of human health, and fascinating developments achieved in the past few decades are impressive examples of an interdisciplinary interplay between medicine, biology and engineering (Kelly et al. 1993; Howaldt et al. 2005). Among the classical products from cells we find viral vaccines, monoclonal antibodies and interferons, as well as recombinant therapeutic proteins

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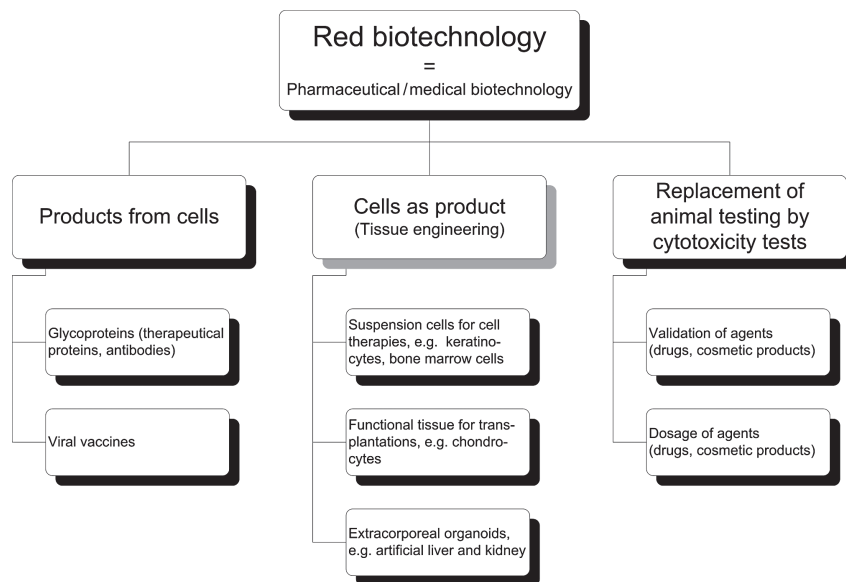
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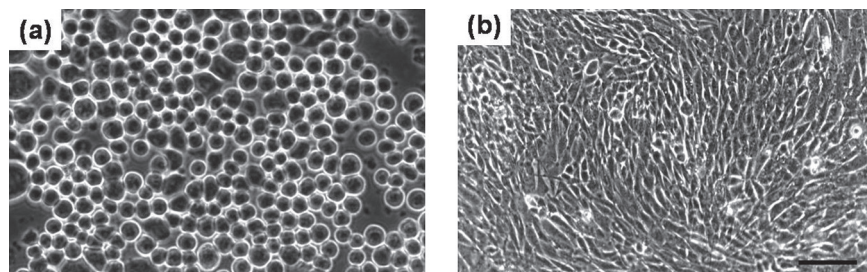
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(Fig. 1.1). Tissue engineering or gene therapy has opened up challenging new areas (Ozturk 2006). “Mammalian cell culture” refers to the cells of a mammalian, isolated from specific tissues (i.e. skin, liver, glands, etc.) and further cultivated and reproduced in an artificial medium (Fig. 1.2) (Butler 2004; Shuler and Kargi 2002). During the cultivation of mammalian cells *in vitro*, outside a living organism, some specific difficulties arise in the extraction of the cells from a “safe” tissue. Slow growth rates with doubling times between 12 and 28 h, low productivity, sensitivity against shear stress due to the lack of a cell wall (Cherry 1993), and complex requirement of growth medium are the challenges in developing techniques for mammalian cell culture. Moreover, many cell lines grow adherent, and a suitable surface for attachment has to be provided for these cells to proliferate. As mammalian cells have originated from multi-cellular organisms, they still hold the genetic program



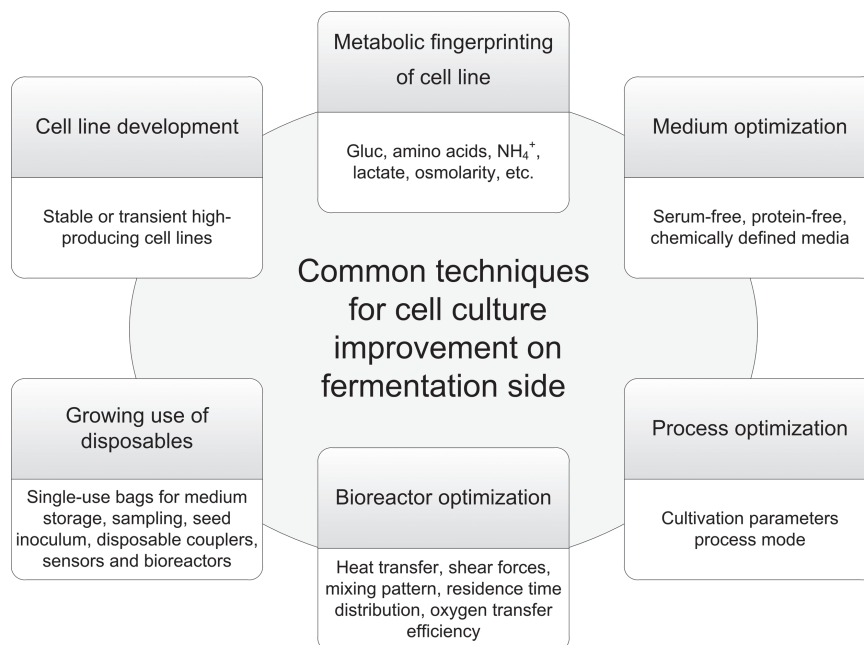
**Fig. 1.1** Application fields of mammalian cells



**Fig. 1.2** Morphology of (a) suspendable and (b) adherent mammalian cells (bar approx. 30  $\mu\text{m}$ )

of inducing their own cell death; a process called “apoptosis” or “programmed cell death” (Cotter and Al-Rubeai 1995; Singh et al. 1994; Al-Rubeai and Singh 1998). This can limit culture productivity in biotechnological processes. Another major problem is the finite life-span of primary cells, which die after several doublings *in vitro*. This problem was solved by transforming primary cells into immortal, “established” or “continuous” cell lines.

Considerable effort has been directed towards the development of mammalian cell culture technology, and appreciable progress has been achieved in the past few decades (Glaser 2001; Kretzmer 2002). Media that used to contain up to 10% serum have been continuously improved, and the cultivation in defined serum-free and even chemically defined, protein-free media is now common for most relevant industrial cell lines. Bioreactors have been developed, which provide the required low shear-stress environment by introducing gentle agitation and aeration with slow moving stirrers in tanks, or designing special aerators for air-lift reactors, or separating the cells from stressful conditions like hollow-fibre, fluidized-bed, and fixed-bed reactors. By using specific fermentation techniques for cell culture improvement (Fig. 1.3) mammalian cells can now be cultured in very large volumes (up to 20,000 L) to provide the needed quantity and quality of the desired product. On the industrial scale the adherent cell lines can be cultivated on micro-carriers (e.g. for vaccine production) or adapted to grow in suspension, e.g. cell lines derived from baby hamster kidney cells (BHK) or chinese hamster ovary cells (CHO). International developments have focused on application of disposables (single-use



**Fig. 1.3** Techniques for cell culture improvement

equipments) in mammalian cell-based processes to reduce the development and process costs, shorten time to market and enable quick process modifications. (Besides disposable bags for storage and sampling, liquid-handling container systems, disposable sensors, filters and couplings, disposable bioreactors have also become increasingly accepted.

Genetic engineering has contributed significantly to the recent progress in this area (Korke et al. 2002; Wurm 2004; Butler 2005). With this technology, functional proteins can be produced by introducing recombinant DNA into cell lines, e.g. chimeric (humanized) antibodies are produced for *in vivo* applications in transfectoma or recombinant CHO cells. New promoters have been developed to enhance productivity, and product titres up to five gram per litre have been reported for some industrial cell lines. By genetically induced proliferation, novel cell lines can now be constructed from primary cells, without losing functionality (Kim et al. 1998; Bebbington et al. 1992).

The following sections provide a basic understanding of the specific requirements of mammalian cells, describe state of the art process technology for cultivation of these cells and give a future perspective. A comprehensive overview on mammalian cell culture technology is given by Ozturk and Hu (2006).

## 1.2 Fields of Application and Products from Mammalian Cells

A detailed overview of the products from mammalian cells is given in (Bebbington et al. 1992; Griffiths 2000). Among “Products from cells” *viral vaccines* (Cryz et al. 2005) against polio, hepatitis B, measles, and mumps for human use and rubella, rabies, and foot-and-mouth-disease (FMD) for veterinary use are important products. Viral vaccines are produced efficiently by cell-based vaccine technology. For this purpose, primary cells, diploid cells or permanent cell lines (e.g. VERO) and recently, even recombinant cell lines are used. A breakthrough for the large-scale production of viral vaccines with anchorage-dependent cells was the development of microcarriers in the late 1960s which permitted cultivation in stirred tanks on thousand-litre scale. New targets for cell-based vaccines are the human immunodeficiency virus (HIV), herpes simplex virus, and influenza. Recent developments include genetically engineered or DNA-vaccines.

*Monoclonal antibodies* (Galfre et al. 2005) have become a valuable tool for diagnostic purposes, as well as therapy. Antibodies synthesized by B-lymphocytes play an important role in the immune system of mammals. Traditionally polyclonal antibodies were isolated from blood samples. In the 1970s Milstein and Kohler developed a technique to generate hybridoma cells producing monoclonal antibodies (Köhler and Milstein 1975). Due to specific binding, monoclonal antibodies are widely used for diagnosis, as tens of thousands of different monoclonal antibodies are available. The importance of monoclonal antibodies as therapeutic agents has evolved only recently, as immunogenic mouse antibodies were replaced

by chimeric, humanized or human antibodies. Areas of application are organ transplantation (OKT3), cancer diagnosis and treatment, rheumatoid arthritis, leukaemia, asthma, and multiple sclerosis. Presently several antibodies are being produced in kilogram quantities (Ozturk 2006). Modern recombinant techniques focus on new antibody formats such as fragmented antibodies (FAB's) or bivalent antibodies, with a broad range of applications.

*Glycoproteins* are another important group of products produced from mammalian cells. Starting with the production of  $\alpha$ -Interferon as an anti-infectious drug by (non-recombinant) Namalwa cells in the late 1970s, a growing number of glycoproteins for treatment of a wide variety of diseases are produced by means of mostly recombinant mammalian cells. Prominent examples are cytokines (e.g. Interferons and Interleukins), hematopoietic growth factors (e.g. Erythropoietin for treatment of anemia), growth hormones, thrombolytic agents (e.g. tissue plasminogen activator [tPA]), coagulation factors (factor VII, factor VIII, factor IX, etc.), and recombinant enzymes (DNase) (Ozturk 2006).

Recombinant proteins may be produced by bacterial, yeast, or mammalian cells. From a technological point of view, hosts such as bacteria or yeast have an advantage as regards the growth rate, final cell density and product concentration. Nevertheless, mammalian cells are preferred for those proteins requiring a specific, human-like glycosylation pattern (Andersen and Goochee 1994; Harcum 2006), which is difficult to obtain in other host systems. Another problem for microorganisms is the maximum size of the protein produced which must be below a molecular weight of approximately 30,000 Da. Further, in contrast to extra cellular release of most proteins produced in mammalian cells, products from microorganisms are often accumulated intra-cellularly in 'inclusion bodies'. This requires a more complex down-streaming. Besides this, for mammalian cells, important parameters such as product yield, medium requirements and growth characteristics (suspendable, shear resistant) have been significantly improved.

Proteins produced in the milk of transgenic animals have begun to compete with "classical" mammalian cell culture (Werner 1998; Young et al. 1997; Garner 1998; Wilmut et al. 1997). The proteins are usually expressed in large titres, over one order of magnitude higher than those obtained from cell cultures. The price for the production in transgenic animals will probably continue to decrease significantly due to the development of more efficient reproduction technologies. These new approaches will significantly decrease the time for the development of a product. The disadvantages of transgenic animals are (i) more sophisticated down-streaming (high protein loads), (ii) long development time, (iii) inability to produce proteins that might impair the health of the animal (e.g. insulin), (iv) higher risk of viral contamination and (v) the possibility of prion contamination (scrapies, BSE).

The field of "Cells as products" includes (i) the development of artificial organs (tissue engineering of liver, kidney) and tissues (skin, cartilage, bone), (ii) the expansion of hematopoietic cells for bone marrow transplantation and (iii) gene therapy. The loss and damage of tissues cause serious health problems (Langer 2000; Griffith and Naughton 2002; Petersen et al. 2003). For example, in the United States, annually almost one-half of the costs of medical treatments are spent on

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