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The Concept of Application of Immobilized and Perfused Mammalian Cells (a Bioreactor Model) in Biomedical Research

H. FARGHALI, L. KAMENÍKOVÁ[1], S. HYNIE

Instiute of Pharmacology, First Medical Faculty, Charles University and [1]Institute of Postgraduate Education in Medicine and Pharmacy, Prague, Czech Republic

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Summary

An overview of the concept of cellular immobilization and perfusion as a small laboratory bioreactor model is presented. The cellular systems currently used may be described as static. This is due to conditions of hypoxia and waste product build-up that affect cell physiology. Cellular immobilization and perfusion is, therefore, expected to maintain the cells for very long periods of time under approximately physiological conditions. A number of applications of immobilized perfused hepatocytes and other cellular systems such as adipocytes and Sertoli cells are described in addition to various other cell lines. Moreover, it is suggested that the bioreactor may have potential use as a bioartificial organ.

Key words

Cell immobilization – Cell perfusion – Bioreactor model

Introduction

Various cellular systems have played a major role in the studies of cell and organ physiology, biochemistry and in the investigations of xenobiotic effects including drugs, chemical carcinogens and other compounds. Freshly isolated cells, primary cell cultures and clonal cell lines are examples of the well established cellular models in various laboratories. In spite of the fact that these models have contributed significantly to our understanding of animal physiology and molecular biology, they have the drawback of being metabolically less active. Indeed, the cellular systems currently used by most workers may be described to be static ones. This is due to the fact that hypoxia and waste product build-up will adversely affect cell physiology in such static cellular systems (Gillies *et al.* 1986.). The ways to alleviate hypoxia is to bubble oxygen intermittently through the cellular suspension or to circulate oxygen over the surface of the culture (Fider and Tolbert 1983). These methods do nothing to remove toxic wastes which accumulate in the medium and can be harmful to relatively fragile mammalian cells (Kilburn and Webb 1968). Static systems are able to maintain cells for a relatively short time. The development of a perfusion method for the cells which normalizes the process of oxygenation and waste build-up removal is, therefore, mandatory and is expected to maintain the cells for very long periods of time and approximately under physiological conditions. Despite the various reported studies utilizing the isolated perfused organs, the perfusion of cells or microoganisms, however, is not straightforward because they flow with the perfusate and block filters used to restrain them. Therefore, the cells have to be immobilized by using an appropriate carrier which ensures cellular perfusion on one hand and the maintenance of cellular functions on the other. In other words, an appropriate bioreactor system that fulfills the forementioned conditions has to be designed.

An immobilized and perfused cellular system potentially has multiple applications in pharmacological and toxicological studies with unparalleled advantages over other cellular systems. In addition, one of the most sophisticated and attractive applications of bioreactors is the study of nuclear

magnetic resonance (NMR) spectroscopy of cultured cells. The latter represents an important extension of this powerful analytical technique that can be used to solve some specific problems that confront the investigator. In this connection, NMR spectrography of cultured cells allows non-invasive and on-line analyses of many biochemical events of definite population(s) of cells (real time measurements). The recent improvements in small laboratory bioreactor systems have, therefore, been successfully presented by NMR spectroscopists beside chemical engineers interested in developing bioreactors (Daly *et al.* 1988, Gillies *et al.* 1993). The present article is an overview of the concept of cellular immobilization and perfusion as a small laboratory biorector model. Other articles in this issue demonstrate the potential applicability of the bioreactor model for studying drug effects and metabolism.

Methods of cellular immobilization

The following methods of cellular immobilization are currently used with a various degrees of success.

1. *Microcarrier beads:* In this method, the cells are cultured on the surface of solid or porous charged plastic beads, the beads are concentrated in an appropriate tube and a fresh medium is superfused over the cells. This method is adequate for anchorage-dependent cells (Ugurbil *et al.* 1981).
2. *Thread technique:* Currently, this is the most popular method because of its simplicity and its applicability to a wide variety of cells (Foxall *et al.* 1984). The cells are mixed with a gel (e.g. agarose gel, calcium alginate, the extracellular matrix known as matrigel) which is allowed to solidify, entrapping the cells in threads. The entrapped cells are placed in a perfusion tube and a fresh medium is superfused over them and nutrients or other xenobiotics are applied to the cells *via* diffusion.
3. *Hollow fibre systems:* In this system, cells are grown to high densities using commercially available or a custom-made hollow fibre system which is suitable for cellular proliferation. These systems are designed to maintain cells for periods of weeks or even months as opposed to days for other systems (Gillies *et al.* 1986, 1993.

It is noteworthy to include, at this point, a perfusion technique which resembles in someway the bioreactor from the functional point of view and may be considered as an alternative. This method applies perfusion of cells in culture dishes as reported recently (Dich and Grunnet 1992). The system accommodates many culture dishes and allows individual medium composition and sampling for each dish.

Applications

Mammalian cells bioreactors have numerous applications in different fields of biomedical research. Some important applications are mentioned below.

1. Design of NMR bioreactor circuits

This is a very convenient technique for prolonged perfusion of mammalian cells as an alternative for perfused organs or using the whole organism. The method is unique and non-destructive for continuous monitoring of many metabolic events that take place in the cells, at a given time. Essentially, it is possible to measure the rates at which various reactions of the pathway take place in a definite population of cells. This can be achieved by a) ^{31}P-NMR spectroscopy to gain information about the intracellular pH and the energy status of cells (some phosphorus-containing drugs can also be used for drug metabolism studies) b) ^{13}C-NMR spectroscopy which is better suited than ^{31}P-NMR for studying the kinetics of metabolic pathways utilizing ^{13}C-enriched substrates; c) ^{1}H-NMR spectroscopy is also a potential powerful tool in cellular metabolic studies in spite of the difficulties associated with high-resolution ^{1}H-NMR studies.

2. Application to various types of cells

a) Hepatocyte immobilization and perfusion

In a recent study we demonstrated that the immobilized perfused hepatocytes are the *in vitro* system worthy of further evaluation (Farghali *et al.* 1992). This system may be useful in the fields of liver cell metabolism in general, liver cell drug metabolism in particular and the response of the liver to putative cytotoxins. Using this model, we have reported a number of findings related to cellular energy status, intracellular pH and intracellular ionic composition. Moreover, the effects of various treatments on many of these physiological parameters have been evaluated (Farghali *et al.* 1991, Gasbarrini *et al.* 1992a,b, 1993).

This kind of experiments, using the hepatocyte bioreactor, potentially offers a unique opportunity to gain new insights on the quantitative aspects of hepatocyte drug biotransformations under physiological conditions. The applicability of such a bioreactor in pharmaco-toxicological research and other fields of molecular cell biology is not only far better than other cellular models but also ethically and economically more acceptable. This stems from the very limited number of animals that are expected to be needed in addition to the ultimate goal of prolonged

use of one single bioreactor. Eventually, a hepatocyte bioreactor may be valuable when supply of hepatocytes is limited. e.g. in the case of human hepatocytes.

b) Sertoli cell immobilization and perfusion

The role of Sertoli cells (SC) in the formation, maintenance and dynamics of the blood-testis barrier (BTB) is well documented. The bioenergetics of immmobilized and perfused SC isolated from rats was assessed using both classical biochemical methods and ^{31}P-NMR spectroscopy. We have demonstrated that isolated, immobilized and perfused SC are stable for prolonged periods (more than 24 h). In addition, this study suggests that SC possess a functional Na^+-Ca^{2+} antiporter and sequester extracellular Ca^{2+} in one or more intracellular compartments (Farghalli *et al.* 1994). The perfusion of SC with ethanol, which acts as a potential gonadal toxin, produced a significant reduction in cellular ATP, O_2 consumption and trypan blue exclusion. Moreover, there was an increase in the intracellular calcium content of SC as a result of ethanol perfusion. These toxic effects of ethanol are not apparently related to acetaldehyde exposure because 4-methyl pyrazole pretreatment potentiated the toxicity of ethanol. This suggests that ethanol is directly toxic for Sertoli cells and its toxicity is not mediated by ethanol metabolism (Farghali *et al.* 1993).

c) Rat adipocyte immobilization and perfusion

Currently the isolated adipocytes from the rat epididymis are being immobilized and perfused in a similar way as hepatocytes and Sertoli cells. The effects of various sympathomimetics and sympatholytics on lipolysis are being followed as a function of drug concentration and time.

3. Immobilized and perfused cells as bioartificial organs

More recently, a novel hepatocyte-loaded hollow fibre bioreactor has been developed as a potential bioartificial liver. The freshly harvested rat hepatocytes were entrapped in a three-dimensional gel matrix within hollow fibres in a perfused bioreactor. Gel entrapment allowed cells to be cultured at high density while maintaining tissue-specific functions. Oxygen consumption, which was evaluated for 7 days, showed that their function was well maintained. Electron microscopy exhibited a distinctive ultrastructure of viable, differentiated hepatocytes, bile canaliculi, intercellular junctions, peroxisomes, abundant mitochondria and glycogen granules. The maintenance of tissue specific function and ultrastructure suggests that this bioreactor configuration has potential as a device to support patients with liver failure (Shatford *et al.* 1992)

Conclusions

Cellular immobilization and perfusion techniques offer more physiological and dynamic methods for studying the diversities of cellular metabolism under normal conditions and after various pharmacological and toxicological manipulations. The method combines the following advantages: 1. the flow through technique of the isolated organs is maintained with the great advantage of using a definite cellular population(s) without disturbing the viability of the cells; 2. the method is unique and non-destructive for continuous monitoring of many metabolic events that take place in the cells at real time.

We have presented several reports which demonstrated that immobilized perfused cells are *in vitro* system worthy of further evaluation. This system may prove to be useful in the fields of cellular metabolism in general, cellular drug metabolism in particular and tissue response to putative cytotoxins. We have carried out a number of applications on immobilized perfused hepatocytes and other cellular system such as adipocytes or Sertoli cells, in addition to various cellular lines that have been reported, by others. Finally, it is suggested that the bioreactor has a potential as a bioartificial organ.

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Reprint Requests

Prof. Dr. S. Hynie, DSc., Institute of Pharmacology, First Medical Faculty, Charles University, 128 00 Prague 2, Albertov 4, Czech Republic.