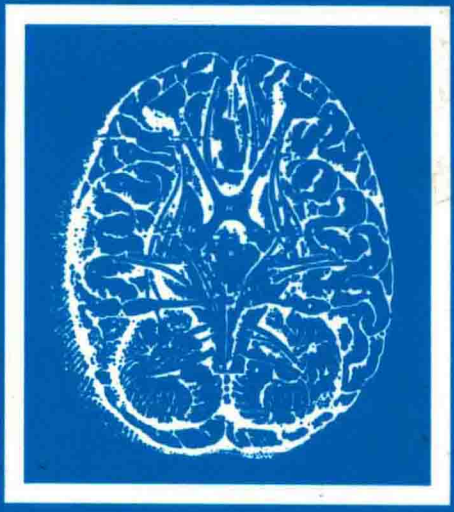
NEUROMETHODS

23

Practical Cell Culture Techniques



Edited by

Alan A. Boulton,
Glen B. Baker, and
Wolfgang Walz

Practical Cell Culture Techniques

NEUROMETHODS □ 23

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Preface to the Series

When the President of Humana Press first suggested that a series on methods in the neurosciences might be useful, one of us (AAB) was quite skeptical; only after discussions with GBB and some searching both of memory and library shelves did it seem that perhaps the publisher was right. Although some excellent methods books have recently appeared, notably in neuroanatomy, it is a fact that there is a dearth in this particular field, a fact attested to by the alacrity and enthusiasm with which most of the contributors to this series accepted our invitations and suggested additional topics and areas. After a somewhat hesitant start, essentially in the neurochemistry section, the series has grown and will encompass neurochemistry, neuropsychiatry, neurology, neuropathology, neurogenetics, neuroethology, molecular neurobiology, animal models of nervous disease, and no doubt many more "neuros." Although we have tried to include adequate methodological detail and in many cases detailed protocols, we have also tried to include wherever possible a short introductory review of the methods and/or related substances, comparisons with other methods, and the relationship of the substances being analyzed to neurological and psychiatric disorders. Recognizing our own limitations, we have invited a guest editor to join with us on most volumes in order to ensure complete coverage of the field. These editors will add their specialized knowledge and competencies. We anticipate that this series will fill a gap; we can only hope that it will be filled appropriately and with the right amount of expertise with respect to each method, substance or group of substances, and area treated.

Alan A. Boulton
Glen B. Baker

Preface

Most cells will survive removal from the natural microenvironment of their in vivo tissue and placement into a sterile culture dish under optimal conditions. Not only do they survive, but they also multiply and express differentiated properties in such a culture dish. A few cells do this in suspension, but most will need some kind of mechanical support substituting for their natural connections with other cells. The surface of a culture dish that might have to be coated is usually sufficient. The recent trend to standardization of conditions and the existence of commercial enterprises with adequate funds and specializing in the needs of scientists were responsible for the tremendous proliferation of cell culture techniques in all fields of research in the last 20 years. No longer does a scientist have to concentrate all his/her efforts on that technology; the new trends make it feasible to employ cell culture techniques as only one of the many methods available in a small corner of a larger research laboratory.

Some areas of research depend more heavily than others on cell culture techniques. Neuroscience is one of the areas that has developed hand in hand with the proliferation of cell culture methodology. Molecular biological aspects, cell differentiation and development, neurophysiological and neurochemical studies, as well as investigations into the nature of various diseases are now to a large extent dependent on the use of cell cultures. Some areas, such as glial cell biology and function, depend almost exclusively on cell culture-based research projects.

In general, most laboratories use cells that have been dissociated from the original tissue and that now form a monolayer or suspension in the culture medium; these are defined as primary cultures. Their properties are related closely to those of the cells from the tissue in vivo and can therefore be used to study cell function in isolation. It is also

possible to select one cell type and obtain one homogeneous culture. If cells from these primary cultures are subcultured after repeated dispersion from the culture dish, and therefore undergo passages through different proliferation cycles, they are called cell lines, and may exhibit loss of some of their differentiated properties. It is then difficult to relate the cultured cells to functional cells in the tissue from which they were derived. Commercially available cell lines are, however, usually well defined, and often have advantages for molecular biological investigations and related problems. Thus, if one is interested in studying the interactions between two or more different cell types in culture, one can either coculture different primary cultures or can use an approach other than one involving dissociated cells. In organ cultures one obtains a three-dimensional culture of undissociated tissue. Here the characteristic architecture of the tissue is largely retained. In organotypic or primary explant cultures a fragment or slice of tissue is used, migration of the different cell types is promoted, and the cells recreate a three-dimensional relationship.

The advantages of the use of tissue culture techniques are obvious. They represent an economical means of obtaining large quantities of homogeneous cells. The isolation of a small population of homogeneous cells facilitates the observation and elaboration of interactions and mechanisms. The environment of the cells can be controlled readily, and all the more so now with the advent of chemically defined media. The cells can be easily defined and the interactions between different cell types can be studied by simply adding more than one cell type to a culture. Not only can specific chemical factors be studied in detail, but the in vitro nature of an experiment means that those can be varied to extreme values. The survival of the whole organism on which the vitality of the studied cell depends and which prevents the use of extreme single parameter settings is not a major problem in the artificial and standardized environment of a cell culture, and larger variations of such factors are possible. Because of increasing public sensitivity to animal rights issues, cell

culture is destined to play an even greater future role in routine testing and toxicology tests. Physiological and biochemical mechanisms can be worked out in culture and subsequent in vivo work can concentrate on experiments of a confirmatory or modulatory nature. Cell culture methodology does not constitute a substitute for future animal research, but it should reduce the need for the use of animals. However, there are some disadvantages associated with the use of cell culture systems. The most important one is that the standardization of the techniques has not yet reached the point where results from different laboratories can be easily compared. We still lack an even rudimentary knowledge of how culture techniques and culture environment change the properties of cell cultures and create differences between in vivo and in vitro properties. In addition, some important parameters, such as cerebral blood flow, cannot be investigated very well in culture.

The present volume of Neuromethods concentrates on the preparation, maintenance, manipulation, and properties of tissue culture systems relevant for neuroscience research. References to the applications of such systems are minimal since many of the other volumes of this Neuromethods series focused on the use of cultured cells, but not on the creation of the cultured cells needed for the described applications. This book attempts to close that gap.

The book is organized into three parts. The first part consists of one chapter that addresses novices who wish to begin cell culturing. It provides valuable information about how to set up a cell culture laboratory, how to sterilize, how to check for contamination, and so on.

The second part of the book examines in detail the many environmental factors that play a role in tissue culturing. The reader is introduced to the use and necessity of cell markers to define the cell culture system used by individual laboratories. This question causes some major scientific controversies, and therefore a sound basis in defining and distinguishing cell types and subtypes is an absolute necessity. Another important issue is the optimal nutritional re-

quirements of the different cell types. The development of chemically defined media for specific cells has opened up a complete new field and research possibilities. It is now possible to study development, expression, and interaction in culture by clearly defining all substances present, without any contributions from unknown factors. Related to this chapter is another one concentrating on growth factors. These are key factors in defining the final architecture of the CNS, synaptic as well as neuronal–glial interactions. Very important tools in cell culture are cell adhesion molecules substituting for the natural adhesion in vivo. They can also be used to select culture conditions for the attachment and therefore selection of specific cell types.

The third and last part of the book will concentrate on the establishment and the properties of some specific brain-derived cell types and systems in culture. This last portion has necessarily been selective. The reader is introduced to three-dimensional organotypic slice explant cultures, which are new and powerful tools with which to study interactions between different cells and cell types. The remainder of this third part of the volume concentrates on primary cultures from more or less homogeneous dissociated monolayer cultures. Neuronal cultures, with special emphasis on the hippocampus, are introduced; they are obviously the backbone of any interest in CNS function. But other cell types are becoming of increasing interest: For example, astrocytes and their subtypes are now of prime neurochemical interest. Oligodendrocytes and their role in myelin formation, as well as in proliferation and differentiation in vitro, are introduced to the reader. Finally, cultures of capillary endothelium derived from cerebral microvessels and their characterization are presented. We anticipate that this volume will suit the needs of the reader who is interested in this ever-expanding area of neuroscience research.

Wolfgang Walz

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