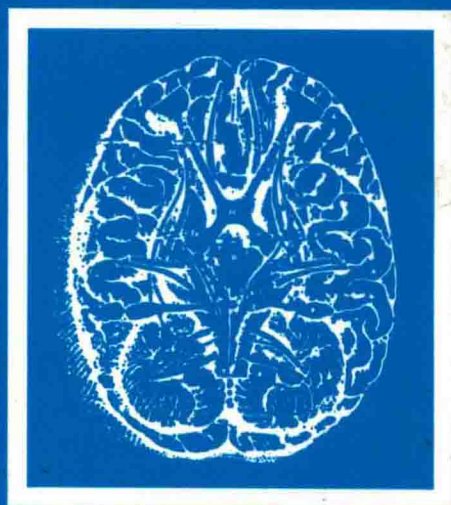


Practical Cell Culture Techniques



Edited by

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

Practical Cell Culture Techniques

NEUROMETHODS □ 23

Edited by

Alan A. Boulton

University of Saskatchewan, Saskatoon, Canada

Glen B. Baker

University of Alberta, Edmonton, Canada

and

Wolfgang Walz

University of Saskatchewan, Saskatoon, Canada



Humana Press • Totowa, New Jersey

© 1992 The Humana Press Inc.
999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

All rights reserved.

No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise without written permission from the Publisher.

Printed in the United States of America.

Library of Congress Cataloging-in-Publication Data

Main entry under title:

Practical Cell Culture Techniques / edited by Alan A. Boulton, Glen B. Baker,
and Wolfgang Walz.

p. cm. — (Neuromethod ; 23)

Includes bibliographical references and index.

ISBN 0-89603-214-0

1. Nervous system—Cultures and culture media. 2. Neurons.

I. Boulton, A. A. (Alan A.) II. Baker, Glen B., 1947– .

III. Walz, Wolfgang. IV. Series.

[DNLM: 1. Neurons—cytology. 2. Tissue Culture—methods. W1
NE337G v. 23 / QS 525 T6155]

QP357.T57 1992

599'.0188—dc20

DNLM/DLC

for Library of Congress

92-1518
CIP

NEUROMETHODS □ 23

Practical Cell Culture Techniques

NEUROMETHODS

Program Editors: Alan A. Boulton and Glen B. Baker

23. **Practical Cell Culture Techniques**
Edited by *Alan A. Boulton, Glen B. Baker, and Wolfgang Walz*, 1992
22. **Animal Models of Neurological Disease, II: Metabolic Encephalopathies and the Epilepsies**
Edited by *Alan A. Boulton, Glen B. Baker, and Roger F. Butterworth*, 1992
21. **Animal Models of Neurological Disease, I: Neurodegenerative Disease**
Edited by *Alan A. Boulton, Glen B. Baker, and Roger F. Butterworth*, 1992
20. **Intracellular Messengers**
Edited by *Alan A. Boulton, Glen B. Baker, and Colin W. Taylor*, 1992
19. **Animal Models in Psychiatry, II**
Edited by *Alan A. Boulton, Glen B. Baker, and Mathew T. Martin-Iverson*, 1991
18. **Animal Models in Psychiatry, I**
Edited by *Alan A. Boulton, Glen B. Baker, and Mathew T. Martin-Iverson*, 1991
17. **Neuropsychology**
Edited by *Alan A. Boulton, Glen B. Baker, and Merrill Hiscock*, 1990
16. **Molecular Neurobiological Techniques**
Edited by *Alan A. Boulton, Glen B. Baker, and Anthony T. Campagnoni*, 1990
15. **Neurophysiological Techniques: Applications to Neural Systems**
Edited by *Alan A. Boulton, Glen B. Baker, and Case H. Vanderwolf*, 1990
14. **Neurophysiological Techniques: Basic Methods and Concepts**
Edited by *Alan A. Boulton, Glen B. Baker, and Case H. Vanderwolf*, 1990
13. **Psychopharmacology**
Edited by *Alan A. Boulton, Glen B. Baker, and Andrew J. Greenshaw*, 1989
12. **Drugs as Tools in Neurotransmitter Research**
Edited by *Alan A. Boulton, Glen B. Baker, and Augusto V. Juorio*, 1989
11. **Carbohydrates and Energy Metabolism**
Edited by *Alan A. Boulton, Glen B. Baker, and Roger F. Butterworth*, 1989
10. **Analysis of Psychiatric Drugs**
Edited by *Alan A. Boulton, Glen B. Baker, and Ronald T. Coutts*, 1988
9. **The Neuronal Microenvironment**
Edited by *Alan A. Boulton, Glen B. Baker, and Wolfgang Walz*, 1988
8. **Imaging and Correlative Physicochemical Techniques**
Edited by *Alan A. Boulton, Glen B. Baker, and Donald P. Boisvert*, 1988
7. **Lipids and Related Compounds**
Edited by *Alan A. Boulton, Glen B. Baker, and Lloyd A. Horrocks*, 1988
6. **Peptides**
Edited by *Alan A. Boulton, Glen B. Baker, and Quentin Pittman*, 1987
5. **Neurotransmitter Enzymes**
Edited by *Alan A. Boulton, Glen B. Baker, and Peter H. Yu*, 1986

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 micro-environment 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

Contributors

- ANN ACHESON • *Regeneron Pharmaceuticals Inc., Tarrytown, NY*
- COLIN J. BARNSTABLE • *Department of Ophthalmology and Visual Science, Yale University, New Haven CT*
- JANE E. BOTTENSTEIN • *Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX*
- JEFFREY R. BUCHHALTER • *Division of Neurology, The University of Pennsylvania, Philadelphia, PA*
- P. DELREE • *Department of Human Physiology and Pathophysiology, University of Liege, Liege, Belgium*
- JEAN DE VELLIS • *University of Southern California at Los Angeles, . Los Angeles, CA*
- MARC A. DICHTER • *Department of Neurology, The University of Pennsylvania, Philadelphia, PA*
- ARACELI ESPINOSA DE LOS MONTEROS • *University of Southern California at Los Angeles, Los Angeles, CA*
- DIANE E. HAROLD • *Department of Physiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*
- LEIF HERTZ • *Departments of Pharmacology and Anaesthesia, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*
- BERNHARD H. J. JUURLINK • *Department of Anatomy, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*
- NIKA V. KETIS • *Department of Anatomy, Queen's University, Kingston, Ontario, Canada*
- P. P. LEFEBVRE • *Department of Human Physiology and Pathophysiology, University of Liege, Liege, Belgium*
- P. LEPRINCE • *Department of Human Physiology and Pathophysiology, University of Liege, Liege, Belgium*
- MARSTON MANTHORPE • *Vical, Inc., San Diego, CA*
- D. MARTIN • *Department of Human Physiology and Pathophysiology, University of Liege, Liege, Belgium*
- G. MOONEN • *Department of Human Physiology and Pathophysiology, University of Liege, Liege, Belgium*

- DAVID MUIR • *Department of Biology, University of California, San Diego, La Jolla, CA*
- BRIGITTE PETTMANN • *Center for Neurochemistry, Strasbourg, France*
- J. M. RIGO • *Department of Human Physiology and Pathophysiology, University of Liege, Liege, Belgium*
- B. ROGISTER • *Department of Human Physiology and Pathophysiology, University of Liege, Liege, Belgium*
- J. SCHOENEN • *Department of Human Physiology and Pathophysiology, University of Liege, Liege, Belgium*
- DAREN URE • *Department of Anatomy and Cell Biology, University of Alberta, Edmonton, Alberta, Canada*
- SILVIO VARON • *Department of Biology, University of California, San Diego, La Jolla, CA*
- WOLFGANG WALZ • *Department of Physiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*
- SUSAN WRAY • *Laboratory of Neurochemistry, NINDS, NIH, Bethesda, MD*

Contents

Preface to the Series	v
Preface.....	vii
List of Contributors	xviii

Basic Techniques for Cell Culturing

Diane E. Harold and Wolfgang Walz

1. Introduction	1
2. Design and Equipping of a Cell Culture Laboratory	2
2.1. Sterile Work Area	2
2.2. Glassware and Instruments	3
2.3. Storage	4
2.4. Incubator Facilities	5
2.5. Laminar Flow Hoods	5
2.6. Source of Water	6
2.7. Cleaning and Sterilization Facilities	7
3. Aseptic Techniques	8
4. Detection of Contamination	9
5. Sterilization and Decontamination	11
5.1. Dry Heat Sterilization	12
5.2. Autoclaving	14
5.3. Sterilization Indicators	14
5.4. Physical Removal: Filtration	15
5.5. Alcohol and Flaming	16
5.6. Chemical Destruction: Use of Antibiotics	17
6. Appendix	17
Acknowledgment	20
References	20

Identification of Cell Types in Neural Cultures

Colin J. Barnstable

1. The Problem of Heterogeneity in Neural Cell Cultures	21
2. Types of Cell Markers Available	22
2.1. Morphological Markers	22
2.2. Enzyme Markers	23
2.3. Pharmacological Markers	26
2.4. Immunological Markers	28
3. The Range of Cell-Type Specific Antibodies Available	28

4. What Antibody Should I Use?	37
4.1. Polyclonal or Monoclonal?	37
4.2. Cell Surface or Cytoplasmic Markers?	37
4.3. Should I Make My Own?	38
5. The Use of Antibodies as Cell Markers in Cultures	39
5.1. Preparation of Cultures for Labeling	39
5.2. Light Microscopic Labeling Methods	41
5.3. Electron Microscopic Labeling Methods	43
5.4. Double-Labeling Procedures	45
6. The Need to Correlate Biochemical and Immunocytochemical Findings	46
6.1. Some Artifacts of Tissue Culture	46
6.2. Commonly Used Biochemical Procedures	49
7. Other Uses of Cell Type-Specific Antibodies	53
7.1. Antibody-Dependent Cell Killing	53
7.2. Antibody-Mediated Positive Selection	54
7.3. Antibodies as Growth Substrates	55
8. Conclusions	57
Acknowledgments	58
References	58

Environmental Influences on Cells in Culture

Jane E. Bottenstein

1. Introduction	63
2. Critical Environmental Parameters	64
2.1. Culture Surface	64
2.2. Basal Culture Medium	65
2.3. Medium Supplements	68
2.4. Physicochemical and Other Variables	69
3. Serum-Free Culture Methods for CNS-Derived Neural Cells	69
3.1. Neuronal Cell Cell Lines	71
3.2. Glial Cell Lines	74
3.3. Embryonic Neurons	74
3.4. Neonatal Type 1 Astrocytes	76
3.5. Neonatal O-2A Lineage Cells	78
4. Conclusion	83
References	83

Detection and Analysis of Growth Factors Affecting Neural Cells

*Marston Manthorpe, David Muir, Brigitte Pettmann,
and Silvio Varon*

1. Introduction	87
1.1. Cells as Probes for "Growth Factors"	87
1.2. Survey of Factor Influences on Neurons and Glia	88
1.3. Principles of Quantification	93
2. Bioassays Using Neurons	96
2.1. Neuronal Survival and Growth Assays	97
2.2. Neurite Promotion Assays	104
2.3. Neurite Inhibition Assays	108
3. Bioassays Using Glial Cells	112
3.1. Morphological Assays	112
3.2. Proliferation Assays	113
3.3. Antiproliferative Assays	117
4. Cell Blot Bioassays	118
4.1. Cell Blot Assays for Neuronotrophic Factors	119
4.2. Cell Blot Assays for Glial Cell Mitogens	121
5. Conclusions and Perspectives	125
References	128

The Role of Cell Adhesion Molecules in Neurite Growth

Daren Ure and Ann Acheson

1. Introduction	139
1.1. How Labeled Pathways Are Created	140
2. Neuronal Cell Culture	142
2.1. The Culture Surface	142
2.2. Preparation of the Culture Surface	143
3. Studying Adhesion Phenomena	153
3.1. Mechanical Interference with Adhesion	154
3.2. Chemical Interference with Adhesion	155
4. Growth Assays	156
4.1. Neurite Initiation and Elongation	157
4.2. Neurite Fasciculation and Branching	158
4.3. Growth Cone Dynamics	158
5. Problems in Interpreting These Types of Studies	159
6. Conclusion	162
References	162

Three-Dimensional Organ Culture Systems

**B. Rogister, J. M. Rigo, P. P. Lefebvre, P. Leprince,
P. Delree, D. Martin, J. Schoenen, and G. Moonen**

1. Introduction	173
2. Organotypic Cultures of the Organ of Corti and of the Spiral Ganglion	174
2.1. Introduction	174
2.2. Methodology	175
2.3. Applications of Organotypic Cultures of the Organ of Corti and of the Spiral Ganglion	178
3. Microexplant Cultures (Cerebellum and Hippocampus)	180
3.1. Introduction	180
3.2. Microexplant Culture of Developing Cerebellum and Hippocampus	181
3.3. An In Vitro System for the Study of Postlesional Gliosis Using Microexplants	183
4. Dissociated Cells in Coculture or Cultured in the Presence of Conditioned Media	186
4.1. Introduction	186
4.2. Purification and Culture Method of Adult Rat DRG Neurons	186
4.3. Plasticity of Neurotransmitter Expression in Adult Rat DRG Neurons	189
5. Aggregate Cultures	191
5.1. Introduction	191
5.2. Organotypic Suspension Culture of Developing Cerebellum	192
5.3. Multicellular Tumor Spheroids	193
6. Conclusion	194
7. In Vivo Veritas?	195
Acknowledgments	196
References	196

Organotypic Slice Explant Roller-Tube Cultures

Susan Wray

1. Introduction	201
2. Slice Explant Roller-Tube Cultures	203
2.1. Ability to Identify Individual Cells	203
2.2. Organotypic Organization	205
2.3. Tissue Types Successfully Cultured	207
3. Slice Explant Protocol	210