

Contents

Foreword V
Rolf Krebs

Preface VII

List of Contributors XVII

Part I Emerging *In-Vitro* Culture Technologies 1

- 1 Intelligent Biomatrices and Engineered Tissue Constructs:
In-Vitro Models for Drug Discovery and Toxicity Testing 3**
*Philip Lazarovici, Mengyan Li, Anat Perets, Mark J. Mondrinos,
Shimon Lecht, Christopher D. Koharski, Paul R. Bidez III,
Christine M. Finck, and Peter I. Lelkes*
- 1.1 Introduction 3
1.2 Intelligent Biomaterials and Scaffolds for Tissue Engineering 4
1.2.1 Synthetic Materials 4
1.2.2 Natural Biomaterials 5
1.3 Fabrication of Scaffolds for Tissue Engineering 7
1.3.1 Electrospinning 7
1.3.2 Controlled Lyophilization 9
1.3.3 Acellularization 10
1.4 Progress and Achievements in Liver Tissue Engineering 11
1.4.1 The Liver 11
1.4.2 Scaffolds for Liver Tissue Engineering 12
1.4.3 Pharmaceutical Applications of Tissue-Engineered Liver Models 15
1.4.4 Conclusions and Novel Trends in Liver Tissue Engineering 16
1.5 Cardiac Tissue Engineering: Cells and Models 16
1.5.1 Cardiac Tissue Engineering 16
1.5.2 Cells used in Cardiac Tissue Engineering 17
1.5.3 Culture Models of Cardiac Tissue-Engineered Constructs 18

Drug Testing In Vitro: Breakthroughs and Trends in Cell Culture Technology
Edited by Uwe Marx and Volker Sandig
Copyright © 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
ISBN: 978-3-527-31488-1

x | Contents

- 1.5.4 Specific Scaffolds Developed for Cardiac Tissue Engineering 20
- 1.6 *In-Vitro*-Engineered Pulmonary Tissue Models: Progress and Challenges 21
 - 1.6.1 Lung Tissue Engineering: The Current State of Play 21
 - 1.6.2 Existing *In-Vitro* Pulmonary Cell and Tissue Culture Biological Models 26
 - 1.6.3 Potential of Alveolar Tissue Models as Disease Models in Pharmaceutical Sciences 27
 - 1.6.4 The Future: Toward Engineered 3D Alveolar Tissue for Cell Therapy and Pharmacological Models 27
- 1.7 *In-Vitro* Models of the Blood–Brain Barrier (BBB) 28
 - 1.7.1 The BBB, a Neurovascular Physiological Unit: The Concept 28
 - 1.7.2 *In-Vitro* BBB Models: Cells and Devices 32
 - 1.7.3 BBB *In-Vitro* Models: From First to Third Generation; the Biological Approach 35
 - 1.7.4 Trends in Tissue Engineering: Realistic *In-Vitro* BBB Pharmacological Models 36
 - 1.7.5 Conclusions for BBB *In-Vitro* Models 38
- References* 39

- 2 An Overview on Bioreactor Design, Prototyping and Process Control for Reproducible Three-Dimensional Tissue Culture 53**
Ralf Pörtner and Christoph Giese
 - 2.1 Introduction 53
 - 2.2 Important Aspects for Bioreactor Design 55
 - 2.3 Culture Systems and Bioreactors Used in Tissue Engineering 57
 - 2.4 The Operation of Bioreactors 59
 - 2.5 3D Systems Used for Drug Testing 62
 - 2.6 Modeling of Bioreactor Systems for Tissue Engineering 62
 - 2.7 The Artificial Immune System 65
 - 2.7.1 Matrices 68
 - 2.7.2 Microenvironment 68
 - 2.7.3 Monitoring 68
 - 2.8 Conclusions 69
- References* 70

- 3 An Overview on Bioelectronic and Biosensoric Microstructures Supporting High-Content Screening in Cell Cultures 79**
Andrea A. Robitzki and Andrée Rothermel
 - 3.1 The Potential of Drug Development and Demand on High-Content Screening Systems 79
 - 3.1.1 Post-Genomics or Proteomics: An Analysis of Manifold Systems and Functional Monitoring of Drugs 79

3.1.2	Pharmaceutical Research and High-Technology Platforms in the Biohybrid Technology Field	80
3.1.3	Synergy of Microchip Technology and Living Cells	81
3.2	Microfabrication Techniques to Generate Miniaturized Chip Components	82
3.3	Microelectrode-Based Techniques for Analyzing Cellular Parameters: Possible Use of Real-Time and HTS of Drugs Without Labeling	85
3.3.1	Impedance Spectroscopy: Screening the Cellular Parameters of Electrophysiologically Inactive Cells	85
3.3.2	Intracellular Recording of Electroactive Cells: Chip-Based, Automated Patch-Clamp Recording	91
3.3.3	Extracellular Recording of Electrically Excitable Cells: Multiple Site Recording of Field Potentials by MEAs	93
3.4	Concluding Remarks: Secondary Screening for Safety and Cost-Effective Drug Testing and Discovery	96
	<i>References</i>	97
4	Novel <i>In-Vitro</i> Exposure Techniques for Toxicity Testing and Biomonitoring of Airborne Contaminants	103
	<i>Amanda Hayes, Shahnaz Bakand, and Chris Winder</i>	
4.1	Introduction	103
4.2	The Inhalation of Air Contaminants	103
4.3	Toxicological Assessment	105
4.4	<i>In-Vitro</i> Toxicological Studies	107
4.5	Applications of <i>In-Vitro</i> Test Methods	107
4.6	<i>In-Vitro</i> Toxicity Endpoints	108
4.7	<i>In-Vitro</i> Toxicity Testing of Air Contaminants	109
4.7.1	Indirect Methods	111
4.7.2	Direct Methods	112
4.8	Conclusions	115
	<i>References</i>	116
Part II Primary Tissues and Cell Lines in Drug Screening/Testing		125
5	Drug Screening Using Cell Lines: Cell Supply, High-Throughput and High-Content Assays	127
	<i>Christa Burger, Oliver Pöschke, and Mirek R. Jurzak</i>	
5.1	Introduction	127
5.2	Cell Lines for HTS	128
5.2.1	Selection of the Most Suitable Cell Line	128
5.2.2	Optimizing Cell Cultivation	130
5.2.2.1	Adherence	130
5.2.2.2	pH and Temperature	130

XII | Contents

5.2.2.3	Media and Additives	131
5.2.2.4	Solvent Tolerance	131
5.2.2.5	Cell Density	131
5.2.3	Optimizing the Reproducibility of Seeding	132
5.2.3.1	Signal Shift	132
5.2.3.2	Edge Effect	132
5.2.4	Cell Production and Plate Delivery	132
5.2.4.1	The Amount of Cells Needed	132
5.2.4.2	Cell Storage	133
5.3	Conventional Cellular Screening Assays	134
5.3.1	General HTS Assay Prerequisites	134
5.3.2	Evaluation of Assay Quality	134
5.3.3	ELISA-Based Assays	135
5.3.4	Radiometric Cellular Assays	136
5.3.5	Reporter Gene Assays	137
5.3.6	Second Messenger Assays	138
5.3.7	Ion Channel Assays	138
5.4	The Definition of High-Content Screening	139
5.4.1	Instrumentation for HCS	139
5.4.2	Reagents (Fluorescent Probes) for HCS	140
5.4.2.1	Low-Molecular-Weight Fluorophores	140
5.4.2.2	Genetically Encoded Reporter for Fluorescence Detection	141
5.4.3	Assays and Target-Based Applications of HCS	142
5.4.3.1	GPCRs	142
5.4.3.2	Kinases	143
5.4.3.3	Other Drug Targets	144
5.4.4	HCS Applications Targeting Generic Cellular Parameters and Morphology	145
5.5	Outlook	146
	References	147
6	Cell Lines and Primary Tissues for <i>In-Vitro</i> Evaluation of Vaccine Efficacy	153
	<i>Anthony Meager</i>	
6.1	Introduction	153
6.2	Measurement of Antigen Expression	155
6.3	Post-Vaccination Testing	158
6.3.1	<i>Ex-Vivo</i> Detection of Antigen-Specific T Cells	160
6.3.1.1	ELISPOT Assay	160
6.3.1.2	Cytokine Capture Assay and Intracellular Cytokine Staining	162
6.3.1.3	Measurement of T-Cell Cytotoxicity	163
6.3.2	Current Knowledge on T-Cell Responses in Vaccine Trials	165
6.4	Future Directions	167
	References	168

- 7 Designer Cells Derived from Primary Tissue and Designed Cell Lines as a Sustainable Cell Source for Drug Discovery and Safety Assessment 177**
Volker Sandig and Ingo Jordan
- 7.1 Introduction 177
- 7.2 Suitability and Limitations of Primary Cells as Physiologic Models 178
- 7.3 Tumor Cell Lines: Sometimes an Alternative 179
- 7.4 Immortalization by Design: Infinite Proliferation and a Differentiated Phenotype? 179
- 7.4.1 Telomerase: the Primary Target in Human Cells 179
- 7.4.2 Inactivation of Rb and p53 Pathways 181
- 7.4.3 Conditional Immortalization 183
- 7.5 Designed Cells in Complex Drug Tests 184
- 7.5.1 Cell Properties Required for Complex Screening Systems 184
- 7.5.2 Complex Designer Cells in Screens 185
- 7.5.3 Viruses and Host Cells in Drug Tests 189
- 7.5.4 Viruses and Designed Host Cells 190
- 7.5.5 Defined Viral and Cellular Pathways and Designed Host Cells 190
- 7.5.6 Virus Field Isolates and Designed Host Cells 192
- 7.5.7 Designed Viruses and Designed Host Cells 193
- 7.5.8 Designed Host Cells Combined 194
- References 196*
- 8 How Human Embryonic Stem Cell Research Can Impact *In-Vitro* Drug Screening Technologies of the Future 205**
André Schrattenholz and Martina Klemm
- 8.1 Introduction 205
- 8.2 First Excursion: Protein Surrogate Biomarker Signatures 208
- 8.3 Second Excursion: Validation 211
- 8.4 Reproductive Toxicology and *In-Vitro* Tests 213
- 8.5 Reproductive Toxicology and hESC 214
- 8.6 Efficacy and Mode of Action Studies: Systems Biology Using Embryonic Stem Cell-Based Screening Systems 218
- 8.7 Conclusions and Outlook 221
- References 222*

**Part III The Use of Human Tissues in Drug Discovery:
Scientific, Ethical, Legal, and Regulatory Environments 229****9 Availability, Standardization and Safety of Human Cells and Tissues
for Drug Screening and Testing 231***Glyn N. Stacey and Thomas Hartung*

- 9.1 Introduction 231
 - 9.2 Availability of Human Cells and Tissues for *In-Vitro* Testing 231
 - 9.2.1 Selecting a Cell-Based System 231
 - 9.2.1.1 Considering the Options for Human Cell-Based Testing 231
 - 9.2.1.2 Establishing a Method Based on an Existing Human Cell Line 232
 - 9.2.1.3 Developing New or Improved Cell Line-Based Techniques 233
 - 9.2.2 Using Donated Human Tissue 233
 - 9.3 Standardization of Cells and Tissues for Testing Purposes 237
 - 9.3.1 Standardization of Primary Cells and Tissues 237
 - 9.3.2 Standardization of Cell Lines 238
 - 9.3.2.1 Challenges for Standardization of Cell Lines 238
 - 9.3.2.2 Achieving Standardization of Cell Lines 239
 - 9.4 Safety Issues 242
 - 9.4.1 Hazards Associated with Human Cells and Tissues 242
 - 9.4.2 Risks from Cell Lines 243
 - 9.5 The Validation of Cell- and Tissue-Based Assays 243
 - 9.6 Conclusions and Future Prospects 245
- References 246*

**10 Ethical Environment and Scientific Rationale Towards *In-Vitro* Alternatives
to Animal Testing: Where Are We Going? 251***Horst Spielmann*

- 10.1 Introduction 251
- 10.2 Legal Framework in Europe for Developing Alternatives to
Experimental Animals 252
- 10.3 Cell and Tissue Culture Systems used in Pharmacology and
Toxicology 254
- 10.4 Drug-Metabolizing Systems 255
- 10.5 Reductions in Experimental Animal Numbers During the Past Decade
in Europe: The Situation in Germany 256
- 10.6 Reducing Animal Numbers in Regulatory Testing by International
Harmonization of Test Guidelines 257
- 10.7 Harmonization of OECD Guidelines for the Testing of
Chemicals 258
- 10.8 Principles of Scientific Validation: The Amden Validation
Workshops 258
- 10.9 Regulatory Acceptance of the Successfully Validated 3T3 NRU
In-Vitro Phototoxicity Test 260

- 10.10 Use of QSAR and Physico-Chemical Exclusion Rules to Predict Skin Irritation Potential 261
- 10.11 Alternative Methods Used in the Development and Safety Testing of Drugs, Biologicals, and Medical Devices 262
- 10.12 The Way Forward 264
 - References* 265

Part IV Summary and Visions 269

11 How Drug Development of the 21st Century Could Benefit from Human Micro-Organoid *In-Vitro* Technologies 271 *Uwe Marx*

- 11.1 Introduction 271
- 11.2 One Hundred Years of *In-Vitro* Culture 272
- 11.3 A Unique Chance Has Been Created by Nature 275
- 11.4 How Do We Explore This Unique Chance? 275
- 11.5 A Roadmap to Enforce New Platform Technologies 276
 - 11.5.1 The Design of Cell Culture Systems and Bioreactors 277
 - 11.5.2 Process Development 277
 - 11.5.3 Human Cell Supply 278
- 11.6 Outlook 280
 - References* 282

Subject Index 283

