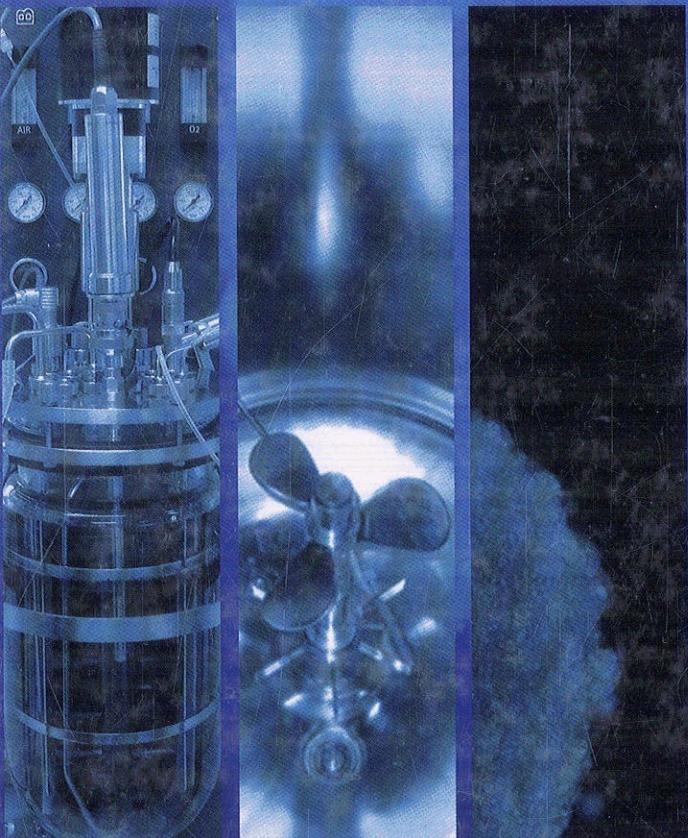


Animal Cell Technology: From Biopharmaceuticals to Gene Therapy

Edited by

Leda R. Castilho, Ângela Maria Moraes, Elisabeth F. P. Augusto and Michael Butler



Contents

Contributors	xiv
Abbreviations	xvi
Foreword	xxxv
1 Introduction to animal cell technology	1
<i>Paula Marques Alves, Manuel José Teixeira Carrondo, and Pedro Estilita Cruz</i>	
1.1 Landmarks in the culture of animal cells	1
1.2 Types of animal cell cultures	3
1.3 Use of animal cells in commercial production	5
1.3.1 Animal cell proteins in human diagnosis and therapy	5
1.3.2 Cell therapy	7
1.3.3 Tissue engineering	8
1.3.4 Gene therapy and DNA vaccines	9
1.3.5 Applications of animal cells in the development of new products	9
1.4 Conclusions	10
References	11
2 Animal cells: basic concepts	13
<i>Patrícia Léo, Adriana Lages Lima Galesi, Cláudio Alberto Torres Suazo, and Ângela Maria Moraes</i>	
2.1 Introduction	13
2.2 Typical structure of an animal cell	13
2.2.1 Plasma membrane	14
2.2.2 Cytoplasm	15
2.2.3 Endoplasmic reticulum	15
2.2.4 Ribosome	16
2.2.5 Golgi complex	16
2.2.6 Mitochondria	16
2.2.7 Lysosome	16
2.2.8 Peroxisome	17
2.2.9 Nucleus	17
2.3 Cell culture	17
2.3.1 Establishing a cell line	17
2.3.2 Cell line maintenance	20
2.4 Cell growth phases	21
2.5 Influence of environmental conditions on animal cell culture	24
2.5.1 pH	24
2.5.2 Osmolality	25
2.5.3 Temperature	26
2.5.4 Oxygen supply	26

2.5.5	Composition and nature of the substratum for cell adhesion	27
2.6	Cryopreservation and storage of cell lines	28
2.7	Culture quality control and laboratory safety	29
2.8	Characteristics of the main cell lines employed industrially	30
2.9	Culture of insect cells	31
2.10	Use of animal cell culture in cytotoxicity assays	32
2.10.1	Culture methods	33
2.10.2	Exposure time and active agent concentrations	34
2.10.3	Recovery time	35
2.10.4	Cytotoxicity evaluation methods	35
	References	36
3	Cloning and expression of heterologous proteins in animal cells	39
	<i>Mariela Bollati-Fogolin and Marcelo A. Comini</i>	
3.1	Introduction	39
3.2	The flow of genetic information and molecular cloning	39
3.3	Elements required for gene expression in eukaryotic cells	40
3.3.1	Transcriptional control elements	40
3.3.2	Translational control elements	42
3.4	Systems for heterologous expression in animal cells	44
3.4.1	Viral vectors	44
3.4.2	Baculoviruses	48
3.4.3	Plasmid vectors	50
3.5	Cell lines and biotechnological processes	54
3.6	Expression in animal cells	54
3.6.1	Transient expression	55
3.6.2	Stable expression	56
3.7	Introduction of DNA into mammalian cells	58
3.7.1	Calcium phosphate co-precipitation method	58
3.7.2	Cationic polymers	59
3.7.3	Lipid-mediated gene transfer (lipofection)	60
3.7.4	Electroporation	60
3.8	Selection markers	61
3.8.1	Morphological changes	61
3.8.2	Biochemical markers and gene amplification	61
3.8.3	Reporter markers	64
3.9	Screening, quantitation, and bioassay methods	66
3.10	Optimizing the initial stage of an animal cell-based bioprocess	66
	References	67
4	Cell metabolism and its control in culture	75
	<i>Paola Amable and Michael Butler</i>	
4.1	Introduction	75
4.2	Energy sources	76
4.2.1	Glucose	76
4.2.2	Glutamine	84
4.2.3	Amino acids	87
4.2.4	Lipids	91

4.3	Metabolic byproducts	95
4.3.1	Lactate	95
4.3.2	Ammonia	96
4.4	Factors affecting cell metabolism	101
4.4.1	Oxygen requirements	102
4.4.2	Carbon dioxide	103
4.4.3	Temperature	103
4.4.4	pH	104
4.5	Conclusions	104
	References	104
5	Culture media for animal cells	111
	<i>Ângela Maria Moraes, Ronaldo Zucatelli Mendonça, and Claudio Alberto Torres Suazo</i>	
5.1	Introduction	111
5.2	Main components of animal cell culture media	114
5.2.1.	Water	114
5.2.2	Glucose	115
5.2.3	Amino acids	116
5.2.4	Vitamins	117
5.2.5	Salts	117
5.2.6	Serum	117
5.2.7	Other components necessary for cell culture	118
5.3	Advantages and limitations of the use of media supplemented with animal serum	121
5.4	Strategies to formulate serum-free culture media	122
	References	125
6	Post-translational modification of recombinant proteins	129
	<i>Michael Butler</i>	
6.1	Introduction	129
6.2	Glycan structures attached to proteins	130
6.2.1	N-glycans	130
6.2.2	O-linked glycans	133
6.2.3	Patterns of glycosylation in nonmammalian cells	134
6.2.4	Glycosylation in animal cells: the effect of the host cell line	137
6.2.5	Culture parameters that may affect glycosylation	137
6.3	Other forms of post-translational modification	138
6.3.1	Deamidation	138
6.3.2	Deamination	139
6.3.3	Glycation	139
6.3.4	Gamma-carboxylation	140
6.3.5	C-terminal modifications	142
6.3.6	Hydroxylation	142
6.4	Conclusions	142
	Acknowledgments	143
	References	143

7	Mechanisms of cell proliferation and cell death in animal cell culture <i>in vitro</i>	147
	<i>Maíra Peixoto Pellegrini, Rodrigo Coelho Ventura Pinto, and Leda dos Reis Castilho</i>	
7.1	Introduction	147
7.2	Cell proliferation mechanisms	147
7.3	Cell death mechanisms: apoptosis and necrosis	151
7.4	Influence of environmental conditions on the induction of cell death	152
7.4.1	Depletion of nutrients and growth factors	152
7.4.2	Oxygen limitation	154
7.4.3	Susceptibility to shear stress	154
7.4.4	Osmolality	155
7.5	Methods of detection of cell death by apoptosis	155
7.5.1	DNA fragmentation	156
7.5.2	Morphological changes	157
7.5.3	Membrane asymmetry	158
7.5.4	Apoptotic proteins	158
7.5.5	Cytochrome C release	159
7.6	Apoptosis suppression by molecular techniques	159
7.6.1	Molecular basis of apoptotic cell death	159
7.6.2	Molecular strategies for apoptosis control	171
7.7	Conclusions and perspectives	173
	References	173
8	Mathematical models for growth and product synthesis in animal cell culture	181
	<i>Elisabeth F.P. Augusto, Manuel F. Barral, and Rosane A.M. Piccoli</i>	
8.1	Introduction	181
8.2	Kinetic analysis of bioprocesses	185
8.2.1	Characteristic kinetic variables	186
8.2.2	Data treatment	190
8.2.3	Phenomena identification	191
8.3	Unstructured and nonsegregated models	192
8.3.1	Classical formulas for cell growth, substrate consumption, and product synthesis	192
8.3.2	Kinetic models for animal cells	199
8.3.3	Parameter fitting in models	209
8.3.4	Model validation	213
8.4	Structured and nonsegregated models	214
8.5	Unstructured and segregated models	215
	References	218
9	Bioreactors for animal cells	221
	<i>Ernesto Chico Véliz, Gryssell Rodríguez, and Alvio Figueroedo Cardero</i>	
9.1	Introduction	221
9.2	Inoculum propagation and small-scale culture systems	221
9.3	Types of bioreactors	224
9.3.1	Homogeneous bioreactors	225
9.3.2	Heterogeneous bioreactors	228
9.4	Modes of operation of bioreactors	234
9.4.1	Batch cultivation	235

9.4.2 Fed-batch cultivation	237
9.4.3 Continuous cultivation	240
9.4.4 Continuous cultivation with cell retention (perfusion)	242
9.5 Aeration and agitation	246
9.6 Scale-up	250
9.7 Economic aspects relevant to bioreactor selection: the productivity factor	252
References	255
10 Monitoring and control of cell cultures	259
<i>Aldo Tonso</i>	
10.1 Introduction	259
10.2 Monitoring and control: basic concepts	259
10.3 Particular characteristics of cell cultures	261
10.4 Main bioprocess variables	261
10.4.1 Temperature	261
10.4.2 pH	262
10.4.3 Dissolved oxygen	263
10.4.4 Cell concentration	265
10.4.5 Other variables of interest	267
10.5 Strategies of control	268
10.5.1 Traditional control	268
10.5.2 Advanced control	270
References	270
11 Animal cell separation	273
<i>Leda dos Reis Castilho and Ricardo de Andrade Medronho</i>	
11.1 Introduction	273
11.2 Separation efficiency	274
11.3 Gravity settling	280
11.4 Centrifugation	281
11.5 Hydrocyclones	283
11.6 Filtration	285
11.6.1 Tangential flow filtration with membranes	285
11.6.2 Dynamic filters	287
11.6.3 Spin-filters	288
11.7 Ultrasonic separation	289
References	291
12 Product purification processes	295
<i>Ângela Maria Moraes, Leda dos Reis Castilho, and Sônia Maria Alves Bueno</i>	
12.1 Introduction	295
12.2 Basic considerations	295
12.2.1 Final application of product	296
12.2.2 Selection of the protein source	297
12.2.3 Protein properties and manipulation	298
12.3 Cell disruption	298
12.4 Protein purification methods	300
12.4.1 Separation processes based on solubility	301
12.4.2 Separation processes based on differences in molar mass	304

12.4.3 Separation processes based on differences in electrical charge	309
12.4.4 Separation processes based on differences in hydrophobicity	313
12.4.5 Separation processes based on specificity of ligands	314
12.4.6 Other developments	319
12.5 Conclusions	323
References	324
13 Quality control of biotechnological products	329
<i>Marina Etcheverrigaray and Ricardo Kratje</i>	
13.1 Introduction	329
13.2 Production of recombinant proteins	331
13.2.1 Control of starting materials	331
13.2.2 Quality control of cell banks	333
13.3 Control of the production process	334
13.3.1 Cultures	334
13.3.2 Purification	335
13.4 Product control	335
13.4.1 Characterization and specification	335
13.4.2 Protein content	336
13.4.3 Amino acids analysis (identification and/or protein content)	336
13.4.4 Protein sequencing (identification)	337
13.4.5 Peptide mapping	337
13.4.6 Electrophoresis	337
13.4.7 Carbohydrate determination	340
13.4.8 Potential impurities and contaminants of biotechnological products	340
13.5 Bioassays	341
13.5.1 Bioassay types	342
13.5.2 <i>In vitro</i> bioassays	343
13.5.3 Experimental design	344
13.5.4 Statistical analysis	345
References	345
14 Regulatory aspects	349
<i>Maria Teresa Alves Rodrigues and Ana Maria Moro</i>	
14.1 Introduction	349
14.2 Good Manufacturing Practices and quality assurance	350
14.3 Regulatory agencies	351
14.4 Harmonization	352
14.5 Premises	353
14.5.1 Clean rooms	353
14.5.2 Biosafety	354
14.6 Cell banks	355
14.6.1 Cell bank qualification	355
14.7 Validation	358
14.7.1 General aspects	358
14.7.2 Biological products	360
14.8 Stability	362
14.9 Clinical trials	362
14.9.1 Preclinical studies	363

14.9.2 Clinical studies	364
14.10 Biogenerics or biosimilars	365
References	367
15 Intellectual property	373
<i>Ana Cristina Almeida Müller and Leila Costa Duarte Longa</i>	
15.1 Introduction	373
15.2 The biotechnology sector	373
15.3 Ethical and moral aspects of research involving genetic engineering	374
15.4 Basic concepts of patentability	376
15.4.1 Discovery versus invention	376
15.4.2 Requirements for the patentability of inventions	377
15.5 Patentable materials	382
15.6 Industrial property and technology transfer offices	384
15.7 Patent and technology transfer specialists	386
15.8 Conclusions	388
References	388
16 Recombinant therapeutic proteins	389
<i>Maria Cândida Maia Mellado and Leda dos Reis Castilho</i>	
16.1 Introduction	389
16.2 Main therapeutic proteins	389
16.2.1 Cytokines	390
16.2.2 Hematopoietic growth factors	392
16.2.3 Growth factors	392
16.2.4 Hormones	393
16.2.5 Therapeutic enzymes	393
16.2.6 Blood coagulation factors	398
16.2.7 Antibodies	399
16.3 Economic aspects	400
16.4 Challenges and future perspectives	402
16.4.1 Formulation and delivery of biopharmaceuticals	402
16.4.2 Characterization of biopharmaceuticals	404
16.4.3 Alternative expression systems	404
16.4.4 Second-generation biopharmaceuticals	405
References	406
17 Monoclonal antibodies	409
<i>Wirla M.S.C. Tamashiro and Elisabeth F.P. Augusto</i>	
17.1 Introduction	409
17.2 Antibodies	411
17.3 Production of monoclonal antibodies	415
17.3.1 Step 1: Immunization	415
17.3.2 Step 2: Fusion and selection of secreting hybridomas	416
17.3.3 Step 3: Hybridoma cloning	417
17.3.4 Step 4: Definition of the isotype of monoclonal antibodies obtained	417
17.3.5 Step 5: Follow-up/later developments	417
17.4 Production of recombinant antibodies	418

17.4.1 Humanized antibodies	420
17.4.2 Human antibodies	421
17.5 Production systems	425
17.5.1 Cell lines	426
17.5.2 Basic conditions for <i>in vitro</i> cultivation	427
17.5.3 Cell metabolism	428
17.5.4 Bioreactors and operation mode	429
References	430
18 Viral vaccines: concepts, principles, and bioprocesses	435
<i>Isabel Maria Vicente Guedes de Carvalho Mello, Mateus Meneghesso da Conceição, Soraia Attie Calil Jorge, Pedro Estilita Cruz, Paula Maria Marques Alves, Manuel José Teixeira Carrondo, and Carlos Augusto Pereira</i>	
18.1 Introduction	435
18.2 Viral replication	436
18.2.1 Adsorption	437
18.2.2 Internalizing and unwrapping the viral particle	437
18.2.3 Structure and organization of viral genomes	437
18.2.4 Production and maturation of viral particles	442
18.3 Production of viral particles by cell culture	442
18.4 Strategies for the production of virus-like particles	447
18.4.1 Advantages of VLPs	448
18.4.2 VLP production technology	448
18.4.3 VLP composition	449
18.4.4 VLP production processes	450
18.5 Development of viruses for DNA vaccines	451
18.6 Perspectives for the evolution of viral vaccine production	452
References	455
19 Bioinsecticides	459
<i>Márcia Regina da Silva Pedrini and Ronaldo Zucatelli Mendonça</i>	
19.1 Introduction	459
19.2 Baculovirus as a bioinsecticide: mechanism of action	460
19.3 Animal cell cultures for baculovirus production	463
19.4 Effect of culture medium, cell line, and virus isolate on biopesticide production	463
19.5 Polyhedra virulence and characteristics	466
19.6 Production of viral mutants in cell culture	467
References	470
20 Cell therapies and stem cells	475
<i>Hamilton da Silva Jr and Radovan Borojevic</i>	
20.1 Introduction	475
20.2 Primary material	476
20.2.1 Stem and mature cells	477
20.2.2 Tissue environment and specific niches	484
20.3 Applications	485
20.3.1 Bioexpansion and biostorage	485
20.3.2 Bioengineering	486

20.4 Conclusions and perspectives	487
References	487
21 Gene therapy	489
<i>Célio Lopes Silva, Karla de Melo Lima, Sandra Aparecida dos Santos, and José Maciel Rodrigues Jr</i>	
21.1 Introduction	489
21.2 Gene therapy	489
21.3 Vectors used in gene therapy	491
21.3.1 Viral vectors	491
21.3.2 Synthetic vectors: plasmid DNA	493
21.4 Principles of gene therapy	497
21.4.1 Replacement or correction of a mutant gene	497
21.4.2 Introduction of a heterologous gene	498
21.4.3 Gene inactivation	498
21.5 Gene therapy and clinical studies	498
21.5.1 The first gene therapy product	501
21.6 Perspectives	502
References	502
Appendix	505
Case study: Mathematical modeling of the monoclonal antibody anti-TNP (trinitrophenyl)	505
Index	507