
Contents

Foreword	xiii
Acknowledgments	xix
Abbreviations	xxi
List of Cell Assays	xxiii
Introduction	xxvii
Chapter 1. Principles and Position	1
1.1. Live cell assay principles.	1
1.2. Application areas	3
1.3. Positioning.	5
1.3.1. Definition and typology of cell tests	6
1.3.2. The regulatory and industrial dimension.	8
1.4. Market	9
1.5. Competitive advantages	12
1.5.1. Cells are live information models.	12
1.5.2. Development: high throughput	13
1.5.3. Development: multiplex analysis	13
1.5.4. Development: miniaturization	14
1.5.5. Development: molecular engineering	14
1.5.6. Development: standardization	14
1.6. Can measurements of cells in culture be extrapolated to effects in the organism?	15
1.6.1. Toxicokinetics	15
1.6.2. Components of the immune system	16

1.6.3. Biotransformation.	16
1.6.4. The macrocellular environment	16
1.7. Limits.	17
1.7.1. Importance of cellular microenvironment	17
1.7.2. Other limits	19
Chapter 2. History and State of the Art	21
2.1. Origins of cell culture	21
2.1.1. Pioneering studies.	22
2.1.2. Alexis Carrel	23
2.1.3. Were Dr Carrel's cells immortal?	25
2.2. The HeLa line and the first applications of cell culture	27
2.2.1. A vaccine against poliomyelitis	29
2.2.2. Cells in space	29
2.2.3. Cell cloning	30
2.3. New cell lines	30
2.3.1. The CHO line	30
2.3.2. An increasing number of cell lines	31
2.4. Cross-contamination.	32
2.5. Cell lines, an ethical issue.	35
2.6. The first generation of cell assays (1969–1983)	37
2.6.1. The karyotype test	38
2.6.2. The MTT assay	39
2.6.3. The NRU test	41
2.7. The first target of regulatory assays: genotoxicity (1983–1986)	42
2.7.1. Ames test (OECD guideline 471)	43
2.7.2. In vitro mammalian chromosome aberration test (OECD guideline 473)	44
2.7.3. In vitro mammalian cell gene mutation test (OECD guideline 476)	45
2.7.4. In vitro sister chromatid exchange assay in mammalian cells (OECD guideline no. 479)	46
2.7.5. DNA damage and repair, unscheduled DNA synthesis in mammalian cells (OECD guideline 482)	47
Chapter 3. Cell Models and Technologies	49
3.1. Fluorescence and bioluminescence.	50
3.1.1. Green fluorescent protein	51
3.1.2. BRET	53
3.1.3. FRET.	55

3.1.4. Other applications of GFP	57
3.1.5. The reporter gene approach	58
3.2. Impedance variation in cell population	60
3.3. Optical signals modified by state of cells	62
3.4. Cellular autofluorescence	65
3.4.1. The case of chlorophyll	66
3.5. The different cell models and culture modes available	67
3.5.1. Immortalized lines	68
3.5.2. Primary cells	69
3.5.3. Three-dimensional cell culture	69
Chapter 4. Loss of Cell Homeostasis: Applications in Toxicity Measurement	71
4.1. What relevant information to use in the living cell?	71
4.2. Lysosomal activity	73
4.3. Redox balance and oxidative stress	76
4.4. Integrity of the plasma membrane	80
4.5. Cellular efflux	84
4.6. Homeostasis of ion exchanges	89
4.6.1. The calcium ion	89
4.6.2. Maintenance of membrane potential	91
4.7. Metabolism and cell respiratory activity	92
4.8. Genotoxicity	95
4.9. Apoptosis	97
Chapter 5. The Replacement of Animal Testing: A Driving Force in Live Cell Assay Development	103
5.1. On the pertinence of <i>in vitro</i> assays	104
5.2. On the pertinence of animal tests	105
5.3. The problem with extrapolation	106
5.3.1. The interspecies barrier	106
5.3.2. The striking example of TGN1412	107
5.4. Toxicological assessment of substances	109
5.5. Irritation and eye corrosion: the long (ongoing) quest for an alternative to the Draize test	111
5.5.1. The CM test	112
5.5.2. Ex vivo approaches	113
5.5.3. 3D culture models	114
5.5.4. Recent attempts and validations	115
5.6. Measurement alternatives for skin absorption, corrosion and irritation (2004–2010)	116

5.6.1. Skin absorption: in vitro method (OECD guideline no. 428)	117
5.6.2. Reconstituted skin models for corrosion and irritation	117
5.6.3. In vitro skin corrosion: human skin model test (OECD guideline no. 431)	118
5.6.4. In vitro membrane barrier test method for skin corrosion (OECD guideline 435)	121
5.6.5. In vitro skin irritation: reconstructed human epidermis test method (OECD guideline no. 439)	121
5.7. The live cell test for phototoxicity measurement (2004)	122
5.8. Assays for endocrine disruptor tracking (2009–2011)	123
5.8.1. Detection of estrogenic agonist-activity of chemicals (OECD guideline 455)	124
5.8.2. H295R steroidogenesis assay (OECD guideline 456)	124
5.9. The four last live cell assays to be validated (2012–2015)	125
5.9.1. Eye corrosion: fluorescein leakage test method (OECD guideline 460)	125
5.9.2. Mammalian cell micronucleus test (OECD guideline 487)	126
5.9.3. ARE-Nrf2 luciferase test method for in vitro skin sensitization (OECD guideline no 442D)	127
5.9.4. Short-time exposure in vitro test method for identifying (1) chemicals inducing serious eye damage and (2) chemicals not requiring classification for eye irritation or serious eye damage (OECD guideline 491)	127
Chapter 6. Regulatory Applications and Validation	129
6.1. Brief history of the validation process in Europe	129
6.2. The validation process of a live cell assay.	130
6.3. Live cell assays adopted by the OECD	132
6.4. The future of regulatory cell tests: the TOX21 and SEURAT programs	134
6.4.1. TOX21, a new paradigm in the assessment of health and environmental risks	134
6.4.2. The SEURAT-1 program (2011–2016)	138
6.5. The REACH regulatory context	139
6.5.1. Assessment approach by weight of evidence (WoE)	140

6.5.2. Up-date on the use of live cell assays under REACH	140
6.5.3. Acute toxicity.	141
6.5.4. Skin corrosion and irritation	142
6.5.5. Eye irritation and severe damage	142
6.5.6. Skin sensitization.	142
6.5.7. Repeated doses (long-term effects)	142
6.5.8. Genotoxicity	143
6.5.9. Reproductive toxicity (reprotoxicity)	143
6.5.10. Carcinogenicity	143
6.5.11. Bioaccumulation and toxicity in fish	144
6.5.12. Long-term toxicity and reprotoxicity in birds	144
6.6. Implementation of the 7th amendment to the Cosmetics Directive	144
6.6.1. Acute toxicity.	145
6.6.2. Eye corrosion and irritation	145
6.6.3. Skin irritation and corrosion	146
6.6.4. Skin sensitization.	146
6.6.5. Genotoxicity	147
6.6.6. Skin absorption.	147
6.7. Food safety and biocides directive	147
6.7.1. Food safety	147
6.7.2. The biocides directive	148
Chapter 7. Cell Signaling: At the Heart of Functional Assays for Industrial Purposes	149
7.1. Membrane receptors, the primary target of drugs	149
7.1.1. Development of the therapeutic target/receptor concept	150
7.1.2. Purification, sequencing and heterologous expression	151
7.1.3. The therapeutic importance of seven transmembrane domain receptors	152
7.2. Second messenger, base unit of the functional live cell assay	153
7.2.1. The second messenger concept	153
7.2.2. Adenylyl cyclase and phosphodiesterase regulate the concentration of cyclic AMP.	155
7.3. The concept of cell transduction.	156
7.3.1. The protein kinase A, the (near) universal target of cyclic AMP.	157
7.3.2. Decrypting the transduction pathways	158
7.3.4. G proteins, the missing link in cell transduction.	160

7.3.5. Connection between transduction and genic expression	161
7.4. The transduction pathways used in the context of live cell assays	162
7.4.1. First level of regulation – activation of the transduction pathway	163
7.4.2. Second level of regulation – desensitization and recycling	164
7.4.3. Third level of regulation – allosteric modulation	165
Chapter 8. Applications in New Drug Discovery	167
8.1. High-throughput screening, the leading market sector for cell assays	167
8.1.1. The role of cell assays in screening programs	169
8.1.2. The contribution of functional cell assays	171
8.1.3. Exploitation of transduction pathways	171
8.2. Measurements in the immediate environment of receptors	173
8.2.1. Assays on receptors	173
8.2.2. β -arrestin activity assays	174
8.3. Measuring cyclic AMP	177
8.3.1. Classic cyclic AMP assays on cellular lysates	177
8.3.2. Cyclic AMP assays on live culture cells	180
8.4. Measurement of the PKC pathway and discrimination of the PKA/PKC pathways	183
8.4.1. IP_3 measurement tests	183
8.4.2. Assays for the measurement of Ca^{2+}	183
8.4.3. Discrimination between the cyclic AMP and IP_3/Ca^{2+} pathways by label-free methods	184
8.5. Measurement of distal signals	185
8.6. Cell assays concerning other therapeutic targets	186
8.6.1. Measurement on ion channels	186
8.6.2. Measurements on receptor tyrosine kinases (RTK)	188
8.7. Pharmacokinetics (ADME) <i>in vitro</i>	191
8.7.1. M for metabolism	192
8.7.2. A for absorption	193
8.7.3. T for toxicity	195
Chapter 9. Impact on Health and the Environment	197
9.1. Patient diagnosis	197
9.1.1. Cytogenetics	198
9.1.2. Diagnosis of tuberculosis	200

9.1.3. Cell assay for the detection of pyrogenic substances	201
9.1.4. Cell assays for predicting efficacy of chemotherapy.	203
9.2. Military programs.	204
9.2.1. Detection and screening of botulinum toxin inhibitors	205
9.2.2. Antibody-based toxin neutralization assays (TNA): application on anthrax and ricin	208
9.2.3. Field measurement of water potability	209
9.3. Pollution and quality of environment	211
9.3.1. The MicroTox assay	211
9.3.2. Mobility of the Daphnia test	212
9.3.3. Fish embryo acute toxicity (FET) test (OECD guideline no. 236)	213
9.3.4. The DR CALUX assay	214
9.3.5. Biomonitoring and field issues	215
Chapter 10. Outlook	219
10.1. Stem cells, an opportunity for the future of cell assays	219
10.2. Organs-on-a-chip	222
10.2.1. Homo chippiens.	224
10.2.2. The contribution of PBPK models	225
10.3. Conclusion	226
Bibliography	229
Index	247