#### ESACT Proceedings

Nigel Jenkins Niall Barron Paula M. Alves *Editors* 

# ESACT

Proceedings of the 21<sup>st</sup> Annual Meeting of the European Society for Animal Cell Technology (ESACT) Dublin, Ireland, June 7-10, 2009





ESACT Proceedings

Volume 5

For further volumes: http://www.springer.com/series/5936 Nigel Jenkins · Niall Barron · Paula M. Alves Editors

Proceedings of the 21st Annual Meeting of the European Society for Animal Cell Technology (ESACT), Dublin, Ireland, June 7–10, 2009



*Editors* Prof. Nigel Jenkins National Institute of Bioprocessing Research & Training University College Dublin Engineering Building, Belfield Dublin Ireland nigel.jenkins@nibrt.ie

Dr. Paula M. Alves Instituto de Biologia Experimental e Tecnológica (IBET) ITQB Oeiras Portugal marques@itqb.unl.pt Niall Barron Dublin City University National Institute for Cellular Biotechnology Dublin Ireland niall.barron@dcu.ie

ISBN 978-94-007-0883-9 e-ISBN 978 DOI 10.1007/978-94-007-0884-6 Springer Dordrecht Heidelberg London New York

e-ISBN 978-94-007-0884-6

Library of Congress Control Number: 2011933343

© Springer Science+Business Media B.V. 2012

No part of this work 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, with the exception of any material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

# Introduction

The 21st ESACT conference was held in the beautiful surroundings of the CityWest Hotel resort in Dublin, Ireland. For the first time in ESACT history the number of participants exceeded 900: a sign of the ever increasing importance of this area. The conference commenced on Sunday June 5th with two sets of parallel workshops on the subjects listed below. An additional workshop was held on Monday lunchtime of the conference.

- 1. Process Analytical Technology (PAT), Quality by Design (QbD) and other recent regulatory developments.
- 2. Innovative media products for the twenty-first century biopharmaceutical industry.
- 3. The impact of high titre media feed-streams on monoclonal antibody purification.
- 4. Advances in genomics and proteomics.
- 5. Stem Cell Technology: new developments and clinical applications.

The first scientific session on Sunday evening included talks on the on-going efforts to gain better understanding of and ways of improving the cell 'factories' that are used to synthesize the 'magic bullets' that are modern day recombinant protein therapeutics. Not that long ago, 1 g/L was considered the holy grail in terms of suspension culture of animal cell systems, yet yields of 10 times that level are achievable nowadays. Despite these gains, researchers in the field strive to further improve on these production platforms by shifting the focus from media and hardware optimization to the molecular mechanisms influencing cell behaviour and productivity in the bioreactor. To this end, methods of applying synthetic biology to explore more novel ways of modifying and utilizing nucleic acid sequence in order to influence protein production or function were presented. In addition, greater insights into the exquisite complexity of the control mechanisms within mammalian cells were given in talks focused on temporal expression of mRNA, protein and microRNAs. The consensus view was that it is likely that the next significant gains

in manipulating cell behaviour and phenotypes may come via engineering of entire networks rather than individual genes.

Sessions 2 and 3 focused on the burgeoning field of stem cells and tissue engineering. From its humble beginnings in egg-based vaccine production, Animal Cell Technology (ACT) has now progressed to such a point that the practical considerations of producing large volumes of cGMP cell therapies are now a reality. A diverse and accomplished panel of speakers described their work on all stages of bringing stem cell therapies from discovery though manufacturing to the potentially life-altering or even life-saving applications in the clinic. Large scale production of stem cells brings its own particular sets of challenges including: maintaining the pluri- or multi-potent state, controlled large scale differentiation, and ensuring product quality. Examples were presented of how some of these cell-based interventions are already proving effective in both animal models and some of the first human trials – a wonderful prospect indeed.

On Tuesday June 9th the day started with a session on Animal Cell Bioprocessing. Eli Lilly & Co. described how a new biotherapeutics facility is under construction near Cork, Ireland alongside an existing chemical pharmaceutical plant. Similar developments are occurring at Pfizer near Cork and together biotherapeutics, chemical therapeutics and medical devices account for almost half of Ireland's exports. Other speakers in this session, which was sponsored by Ireland's National Institute for Bioprocessing Research & Training (NIBRT), covered topics such as using disposable bioreactors at the medium to large scale, and scaled down systems for high throughput screens of media components and environmental conditions. The keynote speaker at the end of this session discussed the development of biogeneric drugs (i.e. follow-on biotherapeutics) that are set to increase market share as the older molecules such as erythropoietin (EPO) come off patent.

Tuesday afternoon also saw the first of two poster sessions, the other being on Wednesday morning. Approximately 300 posters were displayed throughout the conference and prizes for best posters were awarded at the Gala Dinner on the evening of Wednesday June 10th. The final session on Tuesday focused on biotherapeutics, i.e. what types of molecule are under development and how can these be modified for greater potency, safety or half-life?

Linking this session with a Wednesday session on recent developments in vaccines and virology was a keynote talk on the immunogenicity of proteins. Vaccines need to be immunogenic to provoke protective humoral and cell-based responses in humans or animals. However, for non-vaccine biotherapeutics the host immune response must be minimized to avoid raising neutralizing antibodies that may compromise the drug's efficacy. Other topics in the vaccine & virology session included improving influenza virus production, and using the heat shock response to improve the efficiency of host cell line virus production.

This volume contains much of the excellent data presented and discussed at the 21st ESACT meeting.

# Part I Synthetic and Molecular Biology

| MicroRNAs as Potential Engineering Targets for Improvement<br>of CHO Cell Production Phenotypes   | 3  |
|---|----|
| Are Clones Really Unstable?   | 13 |
| An Optimised Transfection Platform for the <i>Epi</i> -CHO Transient<br>Expression System in Serum-free Media   | 19 |
| Using the "OMICS" Technologies as Complementary Tools to<br>Study the Molecular Mechanisms Involved with the Adaptation<br>of Myeloma Cell Line to Protein-Free Medium    | 25 |
| Galectin-3 Over-Expression Enhances Survival and<br>Recombinant Protein Expression in Mammalian Cells   | 31 |
| Metabolomic Analysis of CHO Cultures with Different Growth<br>Characteristics – Development of a Metabolite Extraction<br>Protocol for Suspension Adapted Mammalian Cells | 37 |
| Cell Lines in Four Weeks with the CEMAX <sup>®</sup> System<br>Benedikt Greulich, Karlheinz Landauer, and Andreas Herrmann  | 43 |

| Analysis of Protein Expression via Alternate 3' Untranslated<br>Region (UTR) Signals Through the Use of Site Specific Recombination .<br>Jeff Jia Cheng Hou, Michael Song, Trent P. Munro, and Peter P. Gray      | 47 |
|---|----|
| A Case Study in Clone Screening: A Comprehensive Approach<br>for a Product With High Projected Market Demand  | 53 |
| An Improved Clone Selection Method  | 57 |
| Dissecting the Mechanism of Action of BHRF1 for the<br>Protection Against Apoptosis in MAb-Producing Cell Lines<br>Ernest Milián, Sandra Juanola, Eva Prats, Jordi J. Cairó,<br>Francesc Gòdia, and Joaquim Vives | 61 |
| Qualification of Bench and Pilot-Scale Bioreactors as Modelsof Commercial SystemsAnne Marie Molloy, Patrick Dowling, Mairead Looby, CaitrionaCrawford, Bruce Tangarone, Mary Heenan, and Enda Moran               | 67 |
| Quantification of Polyethylenimine in Transient Gene<br>Expression: On the Way to GMP Compliance  | 71 |
| Implications of Vector Fragmentation and Initial ProductionLevels in Production Cell Line Development Using MTXSay Kong Ng, Wenyu Lin, Rohit Sachdeva, Daniel I.C. Wang,and Miranda G.S. Yap                      | 77 |
| Characterizing the Expression Stability in Different Phenotypes<br>of Recombinant NS0 Myeloma Cell Lines  | 85 |
| A Transient Gene Expression Process with Recombinant<br>Antibody Titer of 0.5 g/L in CHO Cells  | 91 |
| Proteomic Profiling of Temperature-Shifted CHO Cells<br>to Identify Genes that Impact on Both Cell Growth and<br>Recombinant Protein Productivity in Suspension Culture   | 95 |

| Stabilization of Protein Expression in Mammalian CellsEmploying a Toxin/Antitoxin Based StrategyKristina Nehlsen, Jeannette Zauers, Sabrina Herrmann,Hansjörg Hauser, and Dagmar Wirth  | 109 |
|---|-----|
| Novel Cell Lines For Bioprocessing: Friend or Foe?  | 115 |
| Transgene mRNA Levels and Stability are Key Factors<br>to Enhance Transient Gene Expression in CHO DG44 Cells<br>Sarah Wulhfard, Divor Kiseljak, Lucia Baldi, David L. Hacker,<br>and Florian M. Wurm                                   | 121 |
| High Cell Density Transient Gene Expression in HEK 293 EBNA Cells<br>Divor Kiseljak, Gaurav Backliwal, David L. Hacker, Lucia Baldi,<br>and Florian M. Wurm   | 125 |
| Generation of High-Producing CHO Cell Lines by PiggybacTranspositionMattia Matasci, Virginie Bachmann, Fanny Delegrange,Sebastien Chenuet, David L. Hacker, and Florian M. Wurm   | 129 |
| <b>Cellular Proteins in Conditioned Medium Inhibit</b><br><b>Polyethylenimine-Mediated Transfection of CHO Cells</b>  | 135 |
| A New Variant of the Affinity Matrix Method for Identification<br>of High Producing Cells in Mammalian Cell Culture Daniel Landgrebe, Larissa Behr, Pierre Moretti, Johanna Walter,<br>Frank Stahl, Cornelia Kasper, and Thomas Scheper | 139 |
| <b>Novel Strategies for Improving Cell Viability and Production Yield</b><br>Hisahiro Tabuchi, Tomoya Sugiyama, and Satoshi Tainaka   | 143 |
| Part II Stem Cells  |     |
| Regulating in Vitro Motility of Human Mesenchymal Stem<br>Cells with Macrophage Migration Inhibitory Factor (MIF)<br>and a Small-Molecule MIF Antagonist  | 149 |
| Impact of Growth Factors on the Proliferation of Ear           Mesenchymal Stem Cells on Porous Microcarriers           Sébastien Sart, Yves-Jacques Schneider, and Spiros N. Agathos   | 161 |
| <b>Strategies for Pancreatic Differentiation of Pluripotent Stem Cells</b><br>Insa S. Schroeder, Anna Daniel-Wojcik, and Anna M. Wobus  | 177 |

| Contents |  |
|----------|--|
|----------|--|

| <b>Stem Cell Biology: New Applications for Studying Metabolic Diseases</b> .<br>Malte Sgodda, Reto Eggenschwiler, and Tobias Cantz  | 189 |
|---|-----|
| Monitoring of Long-Term Cultivation and Osteogenic<br>Differentiation of Human Umbilical Cord-Derived<br>Mesenchymal Stem Cell-Like Cultures  | 205 |
| <b>cGMP-Compliant Isolation, Expansion and Quality Testing</b><br><b>of Human Bone Marrow-Derived Mesenchymal Stem Cells</b><br>Luca Romagnoli, Ilaria Giuntini, Marta Galgano, Chiara Crosta,<br>Enrico Lucarelli, Davide Donati, Luigi Cavenaghi, and Maria Luisa Nolli | 209 |
| Cancer Research, Molecular and Cell Biology: Look at<br>the Nanotechnology, Based Nanoparticles for Diagnostics,<br>and Therapy. Stem Cell for Treatment of Cancer Diseases<br>Katya Marinova Simeonova   | 213 |
| A Comparative Study of Suspension Cultivation Systems for the<br>Expansion of Undifferentiated Mouse Embryonic Stem Cells<br>Magda Tomala, Sabrina Schmeckebier, Ruth Olmer, Pierre Moretti,<br>Frank Stahl, Ulrich Martin, Thomas Scheper, and Cornelia Kasper           | 219 |
| Part III Tissue Engineering   |     |
| <b>Human Endothelial Cell Lines with In Vivo Physiology</b>   | 225 |
| Quality Control and Cell Line Cross-Contamination: An OldProblem that Continues To FesterJohn R. Masters  | 235 |
| Functional Characterisation of Human Hepatoma Cell LineHepG2 in 3D CultureChristiane Goepfert, Wibke Scheurer, Susanne Rohn,Britta Rathjen, Hans Hoffmeister, and Ralf Pörtner  | 241 |
| GFP-Expressing Bladder Fibroblasts for Applications in Tissue<br>Engineering  | 247 |
| <b>3-Dimensional, Dynamic Cultivations of Human Umbilical</b><br><b>Cord-Derived Cells</b>  | 251 |

| NIH/3T3 Feeder System to Engineer Corneal Epithelial Sheets<br>with Enhanced Positive Progenitor Populations   | 257 |
|--|-----|
| NF-κB Signalling Pathway: Generation and Characterization<br>of a Reporter Cell Line of Human Origin   | 261 |
| Establishing Mammalian Production Cell Lines for Structural<br>Biology by Site-Specific Recombination  | 265 |
| Part IV Advances in Bioprocessing A  |     |
| Regulation of Transferrin Receptor and IGF-I ReceptorNumbers at the Cell Surface Drives Growth and Productivityof Hybridoma CellsAndrew J. Sakko, Kenneth C. Bertram, Sally Grosvenor, ColletteSheahan, Danny Voorhamme, Anthony Simula, and Geoffrey L. Francis | 271 |
| Towards Enhancing Manufacturing Process PerformanceThrough Multivariate Data MiningSalim Charaniya, Huong Le, Keri Mills, Kevin Johnson,George Karypis, and Wei-Shou Hu  | 285 |
| Partial Replacement of Chemically Defined CHO Mediawith Plant-Derived Protein HydrolysatesJames F. Babcock and Amy Antosh  | 295 |
| An Insight into the Physiology of Insect Cells: The Role<br>of Energetic Metabolism on the Cell Density Effect   | 299 |
| <b>Design and Development of a High-Throughput Platform for</b><br><b>Rapid Microbe Identification and Automatic Data Management</b> Björn Breth   | 307 |
| On-line Monitoring of the Live Cell Concentration in Disposable<br>Bioreactors   | 315 |
| The Challenge of Validating a Viable Biomass Probe<br>for cGMP Processes   | 319 |

| Contents |
|----------|
|----------|

| Study of the Effect of High pH and Alkali Addition in aCultivation of Chinese Hamster Ovary CellVéronique Chotteau and Anna Lindqvist  | 323 |
|--|-----|
| Tuning of Dissolved Oxygen and pH PID Control Parametersin Large Scale Bioreactor by Lag ControlVéronique Chotteau and Håkan Hjalmarsson   | 327 |
| Kinetic Analysis of Recombinant BHK Roller Bottle Cultures Frank Deer, Jennifer Mahoney, Abner Correia, and Kevin Stafford   | 331 |
| Manipulation of a Perfusion Process by Medium Optimization Saravanan Desan, Omkar Nandi, Ankur Bhatnagar, and Anuj Goel  | 335 |
| Feed Flow Pulsation in the Separation of CHO Cells inHydrocyclones: Effects of Pressure Drop and Pumphead Typeon Separation Efficiency and Cell ViabilityElsayed A. Elsayed, Leonardo A.G. Ramalho, Leda R. Castilho,and Ricardo A. Medronho | 341 |
| <b>Development of a Biphasic Culture Process for Recombinant</b><br><b>Protein Production in Human CAP Cells</b>   | 345 |
| <b>Crossed Mixture Design and Artificial Neural Networks:</b><br><b>An Efficient Approach to Cell Culture Medium Optimization</b> Guillermina Forno, Caroline Didier, Marina Etcheverrigaray, Héctor Goicoechea, and Ricardo Kratje          | 351 |
| Improving Cell Culture Bioreactor Performance for SensitiveCell Lines by Dynamic Membrane Aeration (DMA)B. Frahm and H. Brod   | 355 |
| Evaluation of Disposable Bioreactor Design Comparedto Reusable Stainless Steel ReactorsGerhard Greller and Ute Noack   | 359 |
| Utilising Scale Model Systems to Optimise Upstream ProcessDevelopment  | 363 |
| <b>Human Platelet Lysates as a Serum Substitute in Cell Culture Media</b> .<br>Caroline Rauch, Elisabeth Feifel, Hans Peter Spötl,<br>Eva-Maria Amann, Harald Schennach, Harald Schöffl,<br>Walter Pfaller, and Gerhard Gstraunthaler        | 369 |
| <b>4 g/L.day: Monoclonal Antibody Volumetric Productivity</b><br><b>in the iCELLis<sup>TM</sup> Disposable Fixed-Bed Bioreactor</b> Jean-Christophe Drugmand, Nicolas Havelange,<br>Florence Collignon, José Castillo, and PA. Girod         | 375 |

| Optimisation of Cell Growth and Recombinant ProteinProduction in Small-Scale Culture Vessels by Using OpticalSensors for On-line Measurement of Dissolved OxygenVolker Jäger, Giannini Apati, and Nadine Konisch   | 379 |
|--|-----|
| A Protocol for Cell Detachment of Vero Cells Grown Under<br>Fully Animal Component Free Conditions and on Cytodex 1<br>Microcarriers   | 383 |
| Simulation and Optimization of Essential Amino Acids<br>in Dynamic Mammalian Cell Culture  | 387 |
| Biomass Sensors in iCELLis <sup>TM</sup> Fixed-Bed Reactors: Data<br>on CHO and Vero Cells   | 391 |
| <b>On-line Monitoring: Animal Cell Cultivation in iCELLis<sup>TM</sup></b><br><b>Fixed-Bed Reactor Using Dielectric Measurements</b> Jean-Christophe Drugmand, Geoffrey Esteban, Naima Alaoui,<br>Nadia Jafâr, Nicolas Havelange, Olivier Berteau, and José Castillo | 395 |
| Change of the Role for JAK/STAT3 in IL-6 Response During<br>Adaptation of Hybridoma Cells to Serum-Free Media  | 401 |
| <ul> <li>Part V Advances in Bioprocessing B</li> <li>Engineering Principles and Cell Culture Performance</li> <li>of Orbitally Shaken Cylindrical Bioreactors</li></ul>  | 407 |
| <b>Dynamic Ranking of Clones with Process Conditions Using</b><br><b>a High-Throughput Micro-bioreactor Platform</b>   | 413 |
| Improved Cell Culture Surface for Biotechnological Research Lara Breth   | 417 |
| Platform Validation of Dissolved Oxygen Ranges for Cell         Culture Processes  | 421 |

| Contents |
|----------|
|----------|

| Study of Growth Factors in Spent Medium for BetterMammalian Cell CultureAkiko Ogawa, Sadaharu Fukui, and Satoshi Terada   | 425 |
|---|-----|
| Process Improvements for Production of a Complex<br>Glycosylated Fusion Protein which Delivers a Two-Fold Increase<br>in Product Titre and Comparable Product Quality Eimear O'Donovan, Patrick Gammell, Mairead Looby,<br>Neysi Ibarra, and Enda Moran | 429 |
| Hydro-Kinetic Modelling of Animal Cell Response to TurbulentMixing in BioreactorsE. Olmos, N. Barbouche, F. Fournier, Fabrice Blanchard,E. Guedon, and Annie Marc   | 433 |
| Near Infrared Spectroscopy as an In-Situ PAT Tool to Monitor<br>Adherent VERO Cell Culture Processes  | 437 |
| VERO Cell Metabolism in Animal Component-Free Media:<br>Influence of Glucose and Glutamine Substitution   | 441 |
| Metabolic Modeling of Drosophila melanogaster Cells Underthe Balanced Growth ConditionR.A.M. Piccoli, F.R.X. Batista, A.M. Moraes,M.F. Barral, M.A. Aguiar, P. Léo, and E.F.P. Augusto  | 445 |
| Mass Transfer Considerations for Scale-Up and Scale-Down<br>of Animal Cell Bioprocesses   | 451 |
| Flow Characterization in Wave Bioreactors Using         Computational Fluid Dynamics         Alper A. Öncül, Yvonne Genzel, Udo Reichl, and Dominique Thévenin  | 455 |
| <b>Effects of Soy Peptone-Supplemented Medium on CHO-320 Cells</b> Jean-François Michiels, Jérémie Barbau, Sébastien Sart, Spiros N. Agathos, and Yves-Jacques Schneider  | 471 |
| Very High Protein Production Levels with a New Transient and<br>Serum-Free Expression System Based on Human CAP Cells<br>Corinna Bialek, Sabine Hertel, Nadine Scholz-Neumann,<br>Ruth Essers, and Gudrun Schiedner                                     | 477 |

| Recombinant Albumin as an Animal-Free Supplement<br>to Enhance Cell Culture Performance   | 481 |
|---|-----|
| The Simcell <sup>TM</sup> High-Throughput Cell Culture System: An         Automated Approach to Integrated Cell Line Selection and         Process Development         Steve R.C. Warr, Yuen-Ting Chim, A. Peter Russo, Brian Benoit,         and Mark Uden | 487 |
| Advanced In Situ Microscopy for On-Line Monitoringof Animal Cell CultureP. Wiedemann, F. Egner, H. Wiegemann, J.C. Quintana, W. Storhas,J.S. Guez, C. Schwiebert, and H. Suhr   | 491 |
| Recombinant Antibody Yield Over 2 g/L by Transient<br>Transfection of HEK 293 EBNA Cells in a Fed-Batch Process<br>Divor Kiseljak, Yashas Rajendra, Gaurav Backliwal,<br>David L. Hacker, Lucia Baldi, and Florian M. Wurm                                  | 497 |
| Rapid Fed-Batch Process Development in SimCell <sup>TM</sup> Zhihua Xiao, Steve Warr, Yuen-Ting Chim, Donald Lee,Erica Wehling, Ekta Goel, David Zhao, and Steve Gorfien  | 501 |
| Automation of Cell Line Development Using the OptiCHOExpression SystemAndrea Salmén, Kristina Lindgren, Lovisa Bylund, Gittan Gelius,Christel Fenge, and Ulrica Skoging-Nyberg  | 507 |
| Modeling of the Evolution of Cellular Density and Nutrient<br>Concentrations in a Fixed-Bed Bioreactor for Its Optimization<br>and Its Scaling-Up   | 511 |
| Valérie Gelbgras, Jean-Christophe Drugmand, and Benoît Haut   | 511 |
| <b>Multi-parameter Process Optimization Using the SimCell<sup>TM</sup> System</b><br>A. Peter Russo, Brian Benoit, Christian Wood, David Jan,<br>and Sadettin S. Ozturk   | 515 |
| Online Measurement of pH and O2 Values During MechanicalStimulation of CellsStefanie Böhm, Solvig Diederichs, Thomas Scheper, Martijn vanGriensven, and Cornelia Kasper   | 519 |
| Part VI Biotherapeutics   |     |
| Reducing Protein Immunogenicity by Design: Deimmunization<br>and Tolerance Induction  | 525 |