

# Miniaturisation - Micro Scale Bioprocess Development

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX: Friday, 24 September 2010

"Miniaturisation and automation of bioprocess development holds great promise in reducing the time and cost to market of new biopharmaceuticals. This meeting aims to highlight recent technologies used in high throughput bioprocess development, from clone selection through to analysis of final product and formulation. A series of expert speakers will describe the development and use of current miniaturisation technologies together with the technical and regulatory hurdles that must be overcome to facilitate wider industrial uptake."

This event has CPD accreditation and will have a discussion panel session.

On registration you will be able to submit your questions to the panel that will be asked by the chair on the day of the event

Meeting Chair - *Professor Gary Lye*, Professor of Biochemical Engineering, University College London

- 8:45 – 9:30 **Registration**
- 9:30 – 9:45 **Introduction by the Chair:** Professor Gary Lye, Professor of Biochemical Engineering, University College London
- 9:45 – 10:15 **Scale Down Approaches to Facilitate CHO Clone Development for High-Level mAB Expression**  
*Dr Ray Field*, Director Cell Sciences, MedImmune, Cambridge, UK.  
With increasingly diverse portfolios of biotherapeutics, more efficient methods for creating and screening high expressing stable cell clones are starting to be used. The ability to screen multiple clones expressing multiple recombinant protein and mAB candidates for each therapeutic project is becoming a requirement in order to identify the appropriate clone / mAB combination for effective drug development. Increasingly, shaking microwell and microbioreactor scale down models are being used to facilitate such clone selections leading to increased predictability of Bioprocessing. Strategies, Issues and case studies will be described to illustrate this approach.
- 10:15 – 10:30 **High Throughput Process Development Technologies: Successful Implementation and Application to Process Improvement.**  
*Dr Jonathan Dempsey*, Life Technologies, UK  
Biopharmaceuticals are an increasingly important class of medicines both in terms of meeting unmet medical needs and commercially. These molecules are manufactured using living organisms and by necessity the processes used are complex, time consuming and costly. In order to increase the availability and reduce the cost of biological medicines novel methods are needed to improve the development phase of these products. Automation of biopharmaceutical development holds great promise in increasing the quantity of these molecules which can be produced and reducing the time and cost to market of these medicines. In this presentation I describe the implementation of two automated cell process development systems, the benefits and limitations of their use and data demonstrating increased throughput and enhanced processes
- 10:30 – 11:00 **The development of a 24/48 vessel automated micro-scale bioreactor and comparison to bench-scale bioreactors**  
*Dr Kenneth Lee*, The Automation Partnership, UK  
Industrial antibody production and many other commercial bioprocesses rely on the selection of the final industrial cell line from hundreds of clones. With such a large number of clones to screen it is inevitable that a large proportion of the clones, possibly over 95%, will not make it to the bioreactor stage: the point at which bioprocesses can be adequately modelled and scale-up principles can be applied. The ideal selection process would be to run a stability check on the system, followed immediately by bioreactor trials. However, the setup and running of bioreactors is laborious, time consuming, and costly. The result is that only the top 1 – 2% of clones are ever evaluated in bioreactors, usually without replication. Static well plates and shake flasks, often used to bridge the gap between stability study and the final clone selection, are inherently different to the bioreactor: static plates have no mechanical input and shake-flasks only have passive control of dO and pH, so high performing clones that have a small process window may be discarded. Bioreactor mimics enabling highly parallel culture and minimising the requirement for labour, provide a solution to this problem. Additionally lower volume systems with reduced footprint decrease costs and minimise the impact on valuable laboratory space. Results from trials of a novel 24-vessel automated micro-scale bioreactor mimic (ambr™) showed very comparable results compared to 5-litre and 10-litre bioreactors. Experiments investigating the growth characteristics in AMBR are very favourable: batch growth shows very tight coefficient of variation (CV) on cell number and viability. Antibody production in fed-batch trials also showed very good comparability with 5-litre and 10-litre bioreactors: a 10 cell clone trial in bioreactors and AMBR showed very similar growth curve profiles, final cell number, and ranking in cell clone productivity. In addition the values for cell productivity rate were also very similar between AMBR and bioreactor.

11:00 – 11:05 **Speakers photo**  
11:05 – 11:30 **Mid-morning break**

11:30 – 12:00 **Microscale to Manufacture for Emerging Vaccine Technologies**

*Dr Jonathan Souquet, Eden Biodesign Ltd., Liverpool, UK*

There has been a recent upsurge in the interest in using viral vectors such as adenoviruses, in the field of viral vectored vaccines and gene therapy. It is estimated that there are currently 377 clinical trials world wide conducted in association with this class of therapeutic product. The recent increase in demand, coupled with the relatively high titres needed for pre-clinical and clinical trials has fuelled the requirement for a new approach to adenoviral production. This presentation describes Eden Biodesign's innovative approach to the rapid development and implementation of a robust, scalable and cost effective purification strategy for the production of adenoviral vectors from a range of suspension cell lines, from bench through to large scale, cGMP clinical manufacture. The design of a 'plug and play' platform process and integration of microscale chromatography techniques have allowed the rapid deployment of an effective adenoviral purification process to meet demand.

12:00 – 12:30 **High-throughput process development technology for design of cleaning-in-place (CIP) protocols for chromatography media**

*Dr Andy Masters, GE Healthcare, UK*

Cleaning-in-place (CIP) of chromatography media is important for the integrity and safety of the final biopharmaceutical product. Efficient and media compatible cleaning procedures also increase the column lifetime and thereby contribute to cost effective processes. We have developed a methodology where numerous cleaning agents and sequences of cleaning steps can be evaluated in parallel using PreDicator™ plates, i.e. 96-well filter plates pre-filled with chromatography media. The PreDicator plates were cycled repeatedly with feed and the cleaning efficiency of a large number of different chemicals and sequences of cleaning steps were evaluated by analyzing the residual amount of proteins on the beads after cleaning. The throughput of the method was maximized by implementing the workflow on a robotic system and by using high-throughput analysis. The correlation between the scale-down screening format and traditional column lifetime studies will be discussed. Process economy calculations comparing different resins and cleaning regimes will also be presented.

12:30 – 13:30 **Lunch and Poster Viewing**

13:30 - 14:30 **Question and Answer Session**

Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day

14:30 - 15:00 **Use of high throughput process development for the process optimisation for Fab antibody fragment purification**

*Dr Dev Baines, ProMetic BioSciences Ltd*

Engineered antibody fragments are of increasing importance as next-generation antibodies for wide ranging applications as biopharmaceuticals and diagnostic tools. The presentation will describe high throughput micro scale downstream process development for purification of Fab antibody fragment using a 96 well plate format tool (PuraPlate™) for process optimisation in conjunction with an adsorbent developed for the capture of antibody fragments (Fabsorbent™ F1P HF). This process was used to optimise for equilibration, sample loading and elution strategies. High throughput experiments allowed for a wide range of buffers and additives to be evaluated in relatively short period of time. The pH of the elution buffer was identified as the most was important parameter for the recovery of the Fab fragments from E. coli lysate with high purity

15:00 – 15:30 **Afternoon Tea/Coffee and Last Poster Viewing**

15:30– 16:00 **Choosing the Correct Tools: Should I DoE it**

*Dr Sam Denby, Oxford Biomedica, UK*

Gene therapy offers great potential to fulfill unmet medical needs. Oxford BioMedica is developing Lentiviral based gene therapy products. The most advanced product in terms of clinical development is ProSavin, a treatment for Parkinson's disease. At present there are no FDA or EMEA licensed gene therapy products, whilst success or failure will ultimately be based on clinical trial results there are a number of unique and interesting manufacturing challenges. Oxford BioMedica are looking at a number of tools including miniaturisation and design of experiments (DoE) to facilitate meeting these challenges rapidly whilst increasing process understanding. This talk will look at some of these methodologies and their application to gene therapy products.'

16:00 – 16:30 **Scale Down Miniaturisation of Process Chromatography with Any Resin with the Atoll 96 array MiniColumn Platform**

*Christian Mueller, Atoll GmbH, Germany*

The 96 array MediaScout® platform from Atoll GmbH offers a uniquely valuable platform for small scale modelling of process resin performance. The system is designed to facilitate selection of resin and optimisation of methods development parameters. Small (200ul packed bed) columns are compression packed to provide similar performance attributes (capacity and resolution) in a convenient parallel array format. Whilst possible to use with PD pipettes or in parallel with centrifugal fluid displacement, the RoboColumn variant is purpose designed to allow robotic operation for fully automated Resin Screening and Methods Development, as well as HT parallel small scale sample handling/preparation. The unique packed column bed offers major performance advantages over other simple resin sampler arrays, and provides a direct small scaledown version with professionally compression-packed process materials. Any particulate resin (>10um) can be specified and each array plate can be populated with resins to suit the proposed experimental purpose. If required, a plate can be populated with up to 96 different resins, though typically each row of 8 is normally configured with 8 replicates of the same resin.

16:30 – 17:00 **Engineering characterisation of miniaturised systems as a basis for rapid bioprocess design and scale-up**

*Professor Gary Lye, Professor of Biochemical Engineering, University College London*

Advances in the miniaturisation of bioprocess unit operations were initially driven by the need for small scale cell culture devices. These are now being matched by novel downstream processing technologies, designed to operate at complementary scales, along with the automation necessary to facilitate parallel and high throughput experimentation. To proceed as rapidly as possible through the stages of bioprocess creation, scale-up and validation, however, requires that the data obtained at each step are quantitative and predictive of larger scales of operation. This presentation will summarise our fundamental understanding of miniaturised bioprocess operations and how this can be used to obtain the greatest benefits from investment in these technologies. Examples will cover aspects of scale-up from cell culture through primary recovery to chromatography highlighting some of the analytical and regulatory challenges that remain to be addressed.

17:00 **Chairman's summing up**

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About the chair

Gary Lye, FICHEM ( <http://www.ucl.ac.uk/biochemeng/staff/lye.htm>), is Professor of Biochemical Engineering and Deputy Head of the Department of Biochemical Engineering at University College London (UCL). He received his PhD in Biotechnology from the University of Reading in 1992 and subsequently held posts in Chemical Engineering at Imperial College London and the University of Edinburgh before joining UCL in 1996. He has broad research interests on the application of microscale and automation techniques to the rapid design, optimisation and scale-up of bioprocesses. At UCL he is Director of the EPSRC Industrial Doctoral Training Centre (IDTC) in Bioprocess Engineering Leadership and a member of the Innovative Manufacturing Research Centre (IMRC) in Bioprocessing. He was also a member of the recent UK Government Industrial Biotechnology Innovation and Growth Team.

About the speakers

*Jonathan Dempsey* is a Process Science Fellow for Invitrogen's PD-Direct Services, joining Invitrogen in 2007 after over 15 years spent working in the Biopharmaceutical industry, where he gained tremendous experience in the development of microbial and cell culture manufacturing processes and cell lines. Jon has also been directly involved in the development of several commercial biotherapeutics. Jon's current responsibilities are to advise and assist European biotechnology companies in developing products and processes for the manufacture of biotherapeutic manufacturing processes.

*Ray Field* is Director of Cell Sciences, in the Development department at MedImmune (formerly Cambridge Antibody Technology) the biologics arm of AstraZeneca. He currently leads development functions including cell line development and upstream bioprocessing including, in-process assays at MedImmune's Cambridge R&D site. He has previously held scientific and leadership positions at Celltech Biologics and also in the research division of (Astra)Zeneca Pharmaceuticals

*Jonathan Souquet* is working as a Senior Downstream Process Development Scientist at Eden Biodesign developing and optimizing purification strategies for a wide range of bio-molecules including recombinant proteins, antibodies, viruses and virus like particles. Jonathan also plays a key role in the subsequent scale up and tech transfer of processes into the GMP processing facilities at the National Biomanufacturing Center and in providing technical support during manufacturing activities. Prior to Eden Jonathan attained a

Ph.D. in the field of chromatography from the department of Biochemical Engineering at the University of Birmingham working within the bio-separations research group. He was also awarded a M.Sc. in Biochemical Engineering from University College London.

*Kenneth Lee* studied in Birmingham university during both his undergraduate and postgraduate. Whilst at Birmingham, Kenneth gained experience in various academic and industrial laboratories including Loughborough University, Keele University, and Smith&Nephew, York. Kenneth Lee started working at The Automation Partnership, a leader in the design and production of automated cell culture since 1989, in 2009 as a Product Development Engineer and Product Specialist in various projects, notably for a new micro-scale bioreactor platform; more information on this system will be given at the presentation. Kenneth has experience in both process design, including industrial bioreactor design, and practical cell biology and cell culture. His experience in both these areas allows him to appreciate problems such as scalability of bioreactor system as well as the practicalities of performing routine cell culture and cell physiology.

*Samuel Denby* is reporting on work performed as a Senior Research Scientist at Oxford BioMedica. Sam joined Oxford BioMedica in 2008 excited by the opportunity to work on the development of manufacturing processes for leading gene therapy products, one of a newer generation of biotherapeutic opportunities. Sam has been working in the Biopharmaceutical field for 10 years, formerly at MedImmune and UCL's Biochemical Engineering departments. Sam's interests are in upstream process development for biotherapeutics, specifically using tools such as scale down models and DoE to understand the impact of process changes on bioprocess performance. Sam has recently joined Becton Dickinson's Advanced Bioprocessing function as Applications and Scientific Manager.

*Christian Müller* studied biology at the University of Constance. He made his diploma in 1999 on the crystallisation of the maltose transporter of *E.coli*, an inner membrane protein. Until 2003 he worked at the University of Cardiff and the University of Constance. From 2004-2007 he worked for AstraZeneca and in 2007 he joined Atoll as a Key Account Manager for middle Europe.

*Dev Baines* is the Technical Director at ProMetic BioSciences Ltd. He joined ProMetic's team in 1996 and has held several positions within the division. Dr. Baines has 20+ years experience in the biopharmaceutical sector along with experience in the research and pre-clinical development of biopharmaceuticals. Dr. Baines has held several positions at GlaxoSmithKline where he was responsible for the Downstream Processing of Proteins and Natural Products. He did post-doctoral research in the Department of Microbiology (Queen Elizabeth College, University of London) and the Department of Biochemistry (St. Bartholomew's Hospital Medical College, London). He received his doctorate in Biochemistry from the University of London in 1979. Dr. Baines is also the author of several peer-reviewed publications and patents

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## POSTERS

### **SCALE DOWN BIOPHYSICAL ANALYSIS FOR BIOPHARMACEUTICAL PRE-FORMULATION**

Simon Webster

Avacta Analytical Limited

Thorough characterisation of candidate protein physical stability early in the development pipeline offers the potential to help select candidates better suited to further development as biopharmaceuticals.

Unfortunately protein material, time and money are often in short supply in early development. This poster introduces an analytical instrument designed to overcome these restrictions which uses optical probes to monitor protein conformational stability and aggregation. The instrument consumes much less protein and is much more rapid than alternative approaches and can investigate up to 48 samples in a single experiment. Example data from a pre-formulation study is presented.

# AUTOMATED EVALUATION OF MICROSCALE LINKED PROCESS SEQUENCES FOR GENERATION OF SCALEABLE BIOPROCESS DESIGN DATA

J.Z. Baboo<sup>1</sup>, J.M. Ward<sup>2</sup>, G.J. Lye<sup>1</sup>, M. Micheletti<sup>1</sup>

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Oxidative bioconversions offer valuable opportunities in industrial pharmaceutical synthesis such as using Baeyer-Villiger monooxygenases for antibiotic synthesis<sup>1</sup>. However, a limiting factor is the identification of scaleable hydroxylation biocatalysts. Coupling high-throughput microscale techniques with automation enables the operation of linked process sequences for faster identification and characterisation of optimal conditions<sup>2</sup>. A fully automated microscale sequence involving fermentation, induction and bioconversion has been developed for the evaluation of whole cell Baeyer-Villiger monooxygenases. The automated approach has been shown to be robust and reproducible over multiple runs producing consistent results on different days. Rapid automated collection of quantitative kinetic data on new bioconversion substrates, substrate concentrations, media formulations and well fill volumes has been achieved. By using a matched oxygen transfer coefficient ( $k_L a$ ) approach both fermentation and bioconversion operations have been successfully scaled up to 2 L<sup>3</sup> and 75 L scale. Current research is focusing on applying the automated microwell sequence for the study of P450 enzyme-catalysed bioconversions<sup>4</sup>.

## References:

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3. Ferreira-Torres C, Micheletti M, Lye G (2005) Microscale process evaluation of recombinant biocatalyst libraries: application to Baeyer-Villiger monooxygenase catalysed lactone synthesis. *Bioprocess and Biosystems Engineering* 28:83-93
4. Hussain HA & Ward JM (2002) Enhanced heterologous expression of two *Streptomyces griseolus* cytochrome P450s and *Streptomyces coelicolor* ferredoxin reductase as potentially efficient hydroxylation catalysts. *Applied and Environmental Microbiology* 69: 373-382

## DEVELOPMENT OF AN AUTOMATED MICROSCALE BIOPROCESS SEQUENCE: APPLICATION TO INCLUSION BODY REFOLDING.

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Inclusion bodies (IBs) are commonly formed in the production of recombinant proteins from *Escherichia coli* (*E. coli*) in the pharmaceutical industry. Active protein is derived from these dense, insoluble inactive aggregates by refolding. Refolding often represents a rate-limiting step in recombinant protein processes, with optimised refold conditions derived empirically. Additionally, a limited understanding has been achieved on the effect of fermentation conditions on the final yield of active protein. Microscale processing techniques can potentially speed up drug development by allowing the study of unit operations using minimal quantities of material and have the potential to be integrated within robotic platforms, thus allowing the parallel study of a large number of process conditions. A refolding process has been developed at the microscale consisting of a series of rapid hierarchical generic assays on an automated platform and has been successfully used to optimise refolding by allowing the high throughput analysis of different refold conditions. The refolding screen is currently being integrated within an automated microscale bioprocess comprising of fermentation, cell harvest, lysis, inclusion body isolation, solubilisation and refolding steps in order to study of the effect of fermentation conditions on inclusion body yields.

## **A HIGH THROUGHPUT, NON-INVASIVE APPROACH FOR FAST SCREENING OF OPTIMAL HOMOGENISATION CONDITIONS FOR A RECOMBINANT *E. COLI***

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Homogenisation represents an important step in the bioprocessings of recombinant proteins and vaccines involving the use of recombinant *E. coli* and yeast cells. Numerous studies showed that it imposes strong effects on the downstream unit operations such as clarification, filtration and chromatography. We here present a high throughput, non-invasive approach for the scale down studies of industrial homogenisation of *E. coli* cells by applying adaptive focused acoustics (AFA) to small sample volumes (1-10 mL) in an automated, batch system (Covaris E210) for high performance sample preparation in glass vials or 96 deep well micro plates. By using a recombinant *E. coli* that produces Fab' in the periplasmic space as a generic model, we find that AFA releases intracellular proteins in a manner that can be described by first order reaction kinetics. The rate constant is linearly correlated with power input, but inversely correlated with sample volume. A key difference between AFA and high pressure homogeniser is the former does not produce high viscosity suspensions when cells break as the latter (Lab 40). However they match each other in viscosity at complete disruption of cells, i.e., by prolonged AFA treatment time or repeated passages by Lab 40. In addition under these conditions it was possible to identify how AFA may be used to prepare a homogenate of similar particle size distribution and centrifugal sedimentation properties as Lab 40. In conclusion, AFA is a convenient way for scale down studies of industrial homogenisation, whose results could be used to predict large scale operations.

## **DESIGN, CHARACTERISATION AND FEASIBILITY OF A MINIATURISED BIOREACTOR TO PERFORM HIGH CELL DENSITY FERMENTATIONS.**

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Use of traditional small scale bioreactors have been limited to initial stages of bioprocess development due to the fact that they lack frequent monitoring and accurate control of critical process variables and incapable of integrating fed-batch operation. As a result the high cell density (HCD) cultivations, essential to commercial bioprocesses, have not been realised. In this study a 25ml miniaturised stirred tank bioreactor (MSBR) has been characterised in terms of its power input and volumetric oxygen transfer rates in order to assess its capability of growing HCD culture and determining adequate scale-up criteria. Engineering characterisation results show scale-up based on equal power input and equal  $k_L a$  are both feasible. An industrial fermentation process of Fab' antibody fragment production was performed and performance of the miniature bioreactor was found to be equivalent to 20 L and 75 L bioreactors in terms of growth and productivity when fermentation was performed at equal specific power input. In order to assess the feasibility of the MSBR to be use for bioprocess development purpose a newly constructed *E.coli* Fab' nuclease strain was characterised in 25ml, 20 L and 75 L bioreactor for growth and productivity. Results showed comparable performance in all three bioreactors when run at equal  $P_G/v$ , suggesting the MSBR could be used in future for efficient bioprocess development.

## **AUTOMATED PARALLEL CHROMATOGRAPHIC SEPARATIONS IN DOWNSTREAM PROCESS DEVELOPMENT.**

Jürgen Friedle and Tim Schroeder

*Atoll GmbH, Ettishofer Straße 10, D-88250 Weingarten, Germany*

A new platform technology has been developed which enables automated column chromatography in a 96 array format. The design allows the user to select any chromatographic material which is packed with due consideration to individual material compression requirements.

Flow in the columns was driven with an 8-piston liquid handler, like in columns individually connected to a one channel stand-alone chromatography system. Fractions from step elution were collected into standard microplates, and then subsequently submitted to a next step for analysis like UV and/ or SDS-PAGE.

In this example a full method development procedure for a therapeutic mAb is described. Loading crude feedstock to a series of different Protein A resins and elution at acidic pH lead to a favourite candidate. This was followed by negative AIEC with 8 different resins. The most suited candidate out of this experiment was optimized regarding protein binding by varying salt concentration and pH.

To obtain information, if a flowthrough or bind/elute step is better suited, a parallel set of experiments was performed with 8 different CIEC materials eluting at increasing salt concentrations.

All experiments were first manually performed with PipetColumns, then transferred to RoboColumns and finally scaled to 10ml LC columns for proof of concept. Final decision on the optimum overall process could be drawn within three days.