ESACT-UK 2013 report by James Budge and Tanya Knight, University of Kent

The 23rd annual ESACT-UK conference took place in the Imago Conference centre in Loughborough. The meeting gave hosted a number of talks covering a large range of topics, as well as a poster session and a large number of trade stands.

DAY 1 – 9th January 2013

Keynote speaker- Perspectives on Cell Culture for Biopharmaceutical Development
Paul G. Varley

The talk gave a review of how have advances in the cell culture process and manufacturing have improved fermenter yield and purification processes. This has resulted in high productivity with reasonable speed and robustness, however predictability of products is still an issue. Developments in transient expression can now allow it to be used routinely for cell line engineering to generate yields of 1-2g/L, which can be used to investigate the predictability of behaviour of drug products. Current technology of disposable reactors also allows for comparable performance at larger scales and increased use of in process analytics to develop or understanding of cells with regard to metabolomics and genomics allowing tailoring of feeds, media, etc to the cell requirements. Future challenges are posed by the increase in novel molecules with new methods of action, which can generate issues with expressibility, stability and heterogeneity. In the future we may also see a shift to alternative cell lines from CHO and more personalised medicines resulting in smaller volumes being required.

Session 1: Upstream Processing

Chair: John Davis, University of Hertfordshire

Design for Success in Early Cell Line Development
Alison Porter; Fujifilm Diosynth Biotechnologies

This talk reviewed how cell line development is on the critical path for first-in-human studies. A change in one aspect of a cell line development process, for example a new host cell line or removal of animal-derived components, can influence the success or failure of both that and other sections in the process. There are a large number of possibilities which can impact on the ability to give ideal cell line characteristics such as vector design, selective pressure and presence of KOZAK sequence. Transfection efficiency was shown to differ between two CHO host cell lines, with values typically varying by between 15 and 20%. This demonstrated the need to optimise the transfection method, for the preferred host cell line, increased the transfection efficiency by up to 6 fold and cell recovery. The effects of buffer and programme conditions on viability and transfection efficiency during electroporation were investigated, as well as the addition of Trehalose or DMSO. Additionally, the effects medium feed versus medium change and the timing of selective pressure application have on growth of recombinant cell lines, and how cloning efficiencies can be increased in animal component-free media were discussed.

Streamlining antibody development using large scale, CHO transient gene expression (TGE) followed by rapid production of CHO stable pools

Peer Heine; MaxCyte

This talk demonstrated the use of the Maxcyte system for both transient and stable production, potentially eliminating the issues of migration from HEK to CHO cell backgrounds which can lead to manufacturing challenges and changes in post translational modifications that can alter the antibody's therapeutic potential. The time line of antibody development can be greatly streamlined using large scale transient gene expression (TGE) directly within CHO cells. CHOS cells can be transfected with >95% transfection efficiency and cell viability using MaxCyte flow electroporation. MaxCyte transiently transfected CHO cells produce antibody titers > 400 mg/L, enabling greater than 1 gram of protein from <3L of culture volume. Using a simple selection protocol, stable clones can be made from MaxCyte transfected CHO cells in 6 weeks which generate antibody titers >1g/L. The Maxcyte system also offers the ability to perform static and flow electroporation, and the option to co-transfect with different vectors.

It's a knockout! Engineering GS CHO Cell Lines

Stuart Martin; Lonza Biologics

This talk demonstrated how the GS-KO of the CHOK1SV host is desirable, by reducing the time to FIH studies, removing toxicity associated with MSX, and eliminating possibility of cells being able to bypass the selection criteria. The methods utilized in the generation of the CHOK1SV GS-KO cell line were discussed. GS-KO was shown to give higher titre and better growth with improved cell line stability and no change in product characteristics. This allows for a new, faster stable pool generation methodology not previously possible with CHOK1SV. All data was generated utilizing new vectors incorporating a mCMV promoter for gene expression.

Session 2: Downstream Processing and Formulation

Chair: Nick Amiss; LGC Standards

High resolution affinity techniques for the purification of therapeutic antibodies

Christopher R Lowe; University of Cambridge

This talk reported the development of synthetic affinity adsorbents for immunoglobulins based on mimicking the IgG-binding domains of Proteins A, L and G. Ligands have been synthesised via triazine and fourcomponent Ugi reactions to generate a substituted peptoidal scaffold mimicking key amino acid residues of Proteins A, L and G. Computer-aided analysis suggests putative binding sites on the respective domains domain of the IgG molecule. *In silico* studies, supported by affinity chromatography in comparison with immobilised Proteins A, L and G, as well as analytical characterisation of the ligand synthesised in solution, indicated the authenticity and suitability of the designed ligand for the purification of immunoglobulins. Preparative chromatography demonstrated the ability of the synthetic ligands to purify IgG and Fab fragments from crude mammalian and yeast cell cultures, under near physiological ionic strength and pH conditions, to yield high purity proteins at a lower cost. This method would potentially allow for the reduction of the number of steps in the downstream process, improving the yields of these steps, reuse of the column and process time/robustness.

Biosimilar process development challenges
Stuart Melville; Eden Biodesign

This talk dicussed the growing market of biosimilars as more blockbuster drugs reach patent expiry. The drivers for this are potential revenue, streamlined product development, increased success rate to market, ability to produce cost effective medicines. The complex high molecular weight structures of biopharmaceuticals, their inherent heterogeneity and dependence on production in living systems gives rise to a number of difficulties during development. Upstream process development can influence the physiochemical characteristics of the product i.e host cell modifications. Media, feed strategy, environmental and physical process will impact on growth and finally method of harvest will affect the product. Therefore need to monitor all the biological, physical and chemical properties which may impact on product safety and quality of the biosimilar.

Determination of protein-protein interactions and their impact on protein liquid formulation behaviour
Robin Curtis; University of Manchester

Therapeutic proteins are often required at high concentrations which can lead to aggregate formation. This discussion focussed on B_{22} protein-protein interactions as a model to investigate a range of solution conditions and the effect on aggregation. Results included data obtained via ACM, differential scanning fluorimetry and charge patch analysis to understand interactions between proteins under varying salt concentration, temperature and ionic charge. This work gives an insight into improved screens for finding the optimal formulation with minimal sample consumption.

Session 3: Early Career Researchers

Chair: Laura Bailey; Eden Biodesign

Profiling the CHO host cell proteome and development of alternate downstream processing of recombinant proteins
L M Chiverton; University of Kent in collaboration with Pall Life Sciences and ChELSI Institute.

Downstream processing and antibody purification is essential for generation of therapeutic proteins. This talk concentrated on the characterisation of host cell protein profiles using 2D HPLC and iTRAQ methods. Over 1700 proteins were identified and studies concluded that although around 98% of total host cell proteins could be used using Protein A chromatography, iTRAQ methods show that trace amounts of around 90% of products can be detected.

Analysis of the activation status of the PI3K/Akt and Ras/MAPK signalling pathways and their roles in the serum-free, suspension adaptation of CHO cells

Robert Whitfield; University of Sheffield in collaboration with ChELSI and Pfizer Research and Development

In order to establish signalling pathway changes that facilitate cell adaptation, differences in PI3K/Akt and Ras/MAPK signalling pathways have been mapped using western blotting and immunoprecipitation. The discussion used a "traffic light" system to highlight upregulation and downregulation of phosphorylation at key sites in both adherent and suspension adapted cells. Abolishing integrin mediated attachment to the extrcellular matrix results in a change in signalling flux towards the PI3K/Akt, as observed in suspension cells.The data suggests the importance of specific signalling intermediates in cell survival and proliferation in differing synthetic environments.

Development of a novel cell cryopreservation platform- benchmarking and DMSO toxicity

Tim Morris; Loughborough University

The importance of cryopreservation was highlighted in this talk with particular focus on toxic properties of DMSO and research into alternatives including glycerol and 1,2-propanediol. Results showed that DMSO was the most effective at retaining high cell viability but also showed that it was more damaging post-thawing. Trehalose and a combination of cryoprotectants were suggested as potential alternatives for DMSO.

Increasing the Yield of Biotherapeutic Recombinant Antibodies from Mammalian Cells by Manipulation of the Signal Sequence Peptide and the Secretory Pathway

Stephanie Shellock-Wells; BBSRC Industrial CASE Studentship with MedImmune Ltd

An overview of N-terminal endoplasmic reticulum (ER) targeting sequences was given illustrating the role of these sequences in antibody secretion overall. The study investigated four endogenous CHO signal sequences and their effect on recombinant protein yields compared to those lacking this sequence. In general these sequences had a positive effect on titre but, interestingly, codon optimising sequences did not necessarily result in improved expression. Evaluation of growth, mRNA stability and productivity were all discussed and highlight the potential for signal sequences to help reduce bottlenecks in the secretory pathway.

DAY 2 – 10th January 2013

Session 4: High Throughput Processes

Chair: Julian Hanak; Cobra Biologics

Automation in cell line development to select suitable clone for manufacturing of biopharmaceuticals
Ulrica Skoging-Nyberg; Cobra Biologics

This talk introduced Cobra Biologics MaxXpress platform for cell line development of mAb producing cell lines using the UCOE (Ubiquitous Chromatin Opening Elements) technology. This process uses DNA sequences from promoters of housekeeping genes prevents gene chromatin from entering the closed state allowing better expression. It includes automated clone screening using the CELLO robotic system, high throughput titer determination using small scale Protein A HPLC and an Ambr microbioreactor system for screening of clones using fed batch processes in expression stability studies. In combination with a toolbox of protein characterisation and bioassays the clone selection can be based on high quality information early in the development chain giving a reduced risk for the project during scale up. The combined process has resulted in improved titre over a small period of time generating a high throughput method.

Using a scale down automated micro-bioreactor system for manufacturing cell line selection
Rahul Pradhan; MedImmune

This talk focused on Cell Line Screening and Phenotypic Stability testing aspects of manufacturing cell line selection for a panel of cell lines expressing a MedImmune recombinant antibody. Conventionally, manufacturing cell lines for recombinant protein production have been screened and tested for phenotypic stability by carrying out multiple fed-batch overgrows in shake flasks (have no control over pH or dissolved oxygen) and assessing recombinant protein production and product quality attributes. The Ambr system and the shake flask method were shown to be comparable over the time of culture, and both methods identified the same top clones and those that were unstable. A micro-bioreactor platform offers several benefits over the shake flask method as extra parameters can be monitored over the culture. A key advantage is the testing of cell lines in a process that closely resembles the manufacturing process, leading to selection of 'manufacturing friendly' cell lines, more suited to bioreactor conditions. This can optimise and streamline bioreactor process development strategies by providing a high quality data set, giving an indication of cell line suitability for large scale manufacturing.

Rise of the machines: Automating the process development workflow
Megan Mason; Lonza Biologics

This talk reviewed the increased use of automation to overcome bottlenecks in the process development workflow, by using scale-down models to improve sample throughput and reduce timelines and implementation of more sophisticated DoE methods. These systems are used to perform repetitive tasks, perform work that may be dangerous to the operator and handle tasks that

require high degrees of accuracy and precision. Within Lonza's Process Development group, automation systems have been incorporated into the cell line construction, bioreactor evaluation, purification development and analytics workflows. Each automation system is operated independently in what is known as "islands of automation" which allows for flexibility while maintaining project segregation when it is required. As similar systems are used early purification methods can be designed and then transferred to later systems for analytics. Within the upstream workflow of Process Development, five times the number of cell lines can now be assessed for productivity by the integration of liquid handling robots in the cell line screening and selection processes, and robotic bioreactor systems have permitted rapid design of bespoke bioreactor processes. To take advantage of the full capacity of these automation systems, high-throughput analytical screen platforms have been introduced to reduce screening from days to hours.

Session 5: Stem Cells and Transgenics

Chair: Karen Coopman; Loughborough University

Facing up to the challenges of *in vitro* blood production
Jo Mountford; University of Glasgow

This talk reviewed the use of human embryonic stem cells to generate red blood cells with the potential to aid with medical supply. Blood Transfusion has become a mainstay of modern medical practice. However problems persist both nationally and internationally in maintaining adequacy of supply, managing the risk of transmission of infectious agents and immune incompatibility between donor and recipient. Human embryonic stem cells (hESCs) have unique properties in that they can be maintained indefinitely in culture in an undifferentiated state and yet retain the ability to form all the cells and tissues within the body. They therefore offer a potentially limitless source from which to generate red cells (RBCs) for use in clinical transfusion. Within the project we have the capability to generate hESC to cGMP grade in compliance with UK regulatory requirements for eventual clinical use. We are able to differentiate these to form haematopoietic progenitor cells (HPC) and to subsequently mature these cells along the erythroid lineage with high efficiency and expansion. The red blood cells produced from hESCs require enucleation. However this process is a current bottleneck and needs to be improved. These red blood cells last approximately 120 days and can be sterilised due to a lack of nuclear material. However, the number of cells required for a therapeutic product, 2 x 1012 cells per unit of RBCs, are vastly in excess to those envisaged for other applications and pose very specific challenges for the development of appropriate bioprocessing and cost models. Genetic manipulation of the hESCs may be possible as any changes to the genetic material will be lost in the end RBCs, as they are enucleated.

Litre-scale expansion and harvest of human mesenchymal stem cells on microcarriers
Qasim Rafiq; Loughborough University

This talk detailed the work which has been undertaken to improve the reproducibility of large scale stem cell production. Human stem cells are promising candidates for cell-based therapies due to their therapeutic efficacy resulting in significant interest in their *in vitro* expansion. However key

issues must be addressed before stem cells can be utilised routinely as cell-based therapies. Amongst them is the need to reproducibly obtain a clinically relevant quantity and quality of stem cells, which can only be achieved by identifying appropriate large-scale culture methods for such cells. At the Centre for Biological Engineering (Loughborough University), we have developed a scalable human mesenchymal stem cells (hMSC) expansion process on microcarriers in a 5 L stirred-tank bioreactor in conjunction with a novel harvesting strategy to successfully retain the quality of the hMSCs after expansion. Viable cell number and daily nutrient/metabolite (glucose and lactate) concentrations were measured. Importantly, a harvesting strategy was also developed to successfully detach a large volume of cells from the cell-microcarrier suspension. The harvested cells retained their immunophenotypic markers, key morphological features and differentiation capacity, outlining the potential to successfully harvest cells of sufficient quantity and quality from microcarriers. It is believed that this is the first instance in which both the successful culture of hMSCs on microcarriers as well as the subsequent harvesting of the cells has been performed at this scale.

The role of transgenics in drug discovery
Colette Johnston; Crescendo Biologics Ltd

The benefits of using transgenic cell lines were outlined with particular focus on mouse models to obtain humanisation to express human antibody fragments i.e. native mouse antibodies have been knocked out and replaced with human antibodies. The resulting cell lines have a robust response to immunogens, antibodies bind with high affinity and have a slow unbinding rate and fragments are thermostable. Transgenic mice have a diverse repertoire and undergo *in vivo* maturation.

Keynote speaker - The CHO cell factory: Engineering the shift from screening to design
David James; University of Sheffield

Cellular heterogeneity can result in a vast range of phenotypes some of which may be desirable characteristics for recombinant protein production. The talk described different factors that can lead to cellular variations including growth rate and responses to cellular stresses and mitochondrial variability which may account for a large amount of overall variability. The engineering paradigm to “measure, model, manipulate and manufacture” was discussed in relation to the cell line development protocols which currently use vast screening processes to obtain high producing cell lines as opposed to a structured method to design and engineer cell lines with desirable phenotypes. It was suggested that computational tools, synthetic biology and genomic analysis could be valuable in reducing the unpredictable nature of cell heterogeneity thus allowing for more direct cell engineering to accomplish cell factories with advantageous phenotypes.

Session 6: 'Omics

Chair: Ben Sykes; BBSRC

MicroRNAs: Engineering tools to enhance CHO cell performance
Paul Kelly; Dublin City University.

MicroRNAs have properties in regulating gene expression at the post transcriptional level. It is suggested that they can potentially target 30% of protein coding genes and they consist of intergenic, intronic and exonic miRNAs. They are highly conserved in CHO cells and are associated with different phenotypes such as growth rate. A shift in temperature causes a shift in miRNA profiling and this study resulted in the identification of miRNA (miR-7) which, when overexpressed in CHO cells, induces a temperature shift phenotype i.e. a reduction in cell density and an increase in specific productivity.

Using metabolite profiling data to make a difference to CHO cell bioprocesses
Alan Dickson; University of Manchester

Processes including cell & medium separation, quenching process and appropriate extractions were outlined as valuable techniques used to profile metabolites. These were used to investigate relationships between feeding, cellular metabolism and desirable phenotypes including growth and productivity. Glycerol, lactate pyruvate and sorbitol were all used as markers of metabolism and the identification of differing levels allows tailoring of feeds in response to cellular requirements and determines the efficiencies by which cells are using feeds for productivity and growth. Metabolic profiling is also useful to inform feeding times.

Building genome-scale models of metabolism
Paul Dobson; University of Sheffield

This discussion focussed on computer based methods and models to identify metabolic targets that could be used to tweak the cell to obtain desirable metabolic phenotypes. The CHO metabolic network (http://cho.sf.net) and knockout libraries were highlighted as useful tools to identify, predict and simulate cellular responses to these tweaks (e.g. knockouts, knockdowns etc.). Although *saccharomyces* yeast has been used as a model to provide data for approximately 2,500 genes, statistical maps are not yet complete enough so practical data is required to aid these models.