THE ESACT NEWSLETTER

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Editorial – Happy New Year

Dear Readers,

It is with great sadness that we have to report the passing away of our dear friend and bright young scientist, Dr. Lisa Hunt. A few days after completing the last newsletter, we heard this dreadful news and would like to dedicate this issue to her, written by her thesis supervisor, Prof. Florian Wurm.

Lisa, I think would have wanted us to move on and make significant impact on the globe through this Society. We therefore invite enthusiastic and energetic partners to volunteer to fill her position as co-editor. As inducements for younger scientists, there is an honorarium and free attendance to ESACT meetings as well as visibility within the ESACT community.

As many of you are aware, we are moving into an era where large scale manufacturing of biotherapeutics will become critical to the success of our industry.

Hence in this packed New Year issue, we begin with the Chairman's note about the Expression of Interest for training in **Industrial Animal Cell Technology** and the ESACT 18th Meeting; There is an introduction to the new UK National Stem Cell Bank. We have an invited review about **large scale animal cell culture** from Birmingham University and fascinating reports from industry on the use of **Microcarrier Technology** and some perspectives on purification from the last ACS meeting.

Finally, we begin the year with some jokes to liven things up and welcome new members to our learned society. Please also note the ***Secretariat's new e-mail***.

Looking forward to your feedback

Your Chief Editor, Steve Oh

In memoriam
Dr. Lisa Hunt
1973-2002

Lisa smiling – a warm and embracing smile. Lisa was a person who would approach anyone without hesitation. She was direct and friendly but clear and sometimes demanding. Lisa joined my laboratory in 1997 to pursue a doctorate. She had been living in Switzerland since 1994, doing a masters thesis, while being an exchange student from the University of Iowa. Her French was impeccable; something I was always jealous about.

Within a short time, Lisa became the center of "fun" among the staff and students of the laboratory, throwing out ideas of things to do and then getting everybody on board to actually do it. Of course she also took charge of things that had to be initiated, improved, or cared for to enhance the general organization of the lab. Nobody had a chance to resist, even the most senior collaborators.

With this same drive Lisa attacked her thesis work. She wanted to do things, get them done fast, and go on to something new. Initially, we had a few difficulties because, in her opinion, I was supposed to be more direct in my guidance. However, she rapidly learned to produce wonderful data. Within months one could see her rapid transformation and maturation as a scientist. This process led to a few widely quoted papers, all featuring her favorite "green cells". For Lisa, achievements were a natural thing, not something she needed to brag about. She collected them with a very relaxed and unassuming attitude.

After finishing her PhD she looked for a job and had several offers, one from a small start-up in Bern. She decided on this one over an offer from a more stable house, even though she had to wait for 3 months to begin because of funding problems. When she asked me whether she could stay for this period, I did not hesitate. During these three months she attacked a scientific problem that we had been thinking about for some time, but we did not have the technical means for solving it. Lisa took the challenge and rapidly collected data from well-planned experiments that I believe will have a big impact on animal cell technology. She identified and optimized conditions that allow the interruption of cell culture at almost any time without freezing cells. Interruption or "pausing" can be done at any time and can last for a single day or for several weeks. Culture vessel with cells (adherent or suspended) are transferred from the standard 37ºC environment to one of reduced temperature (between 4ºC and 28ºC) in which gas exchange is prevented. After having assembled most of the necessary data we started to work on a publication. Unfortunately, and to our most profound shock, Lisa did not have the chance to finalize this work. On a weekend trip to the Mont Blanc area early in September she slipped, fell and injured her head in such a severe way that she would not recover. She passed away on Sept. 16th, 2002.

The ESACT community has lost a very good friend and somebody who wanted us to grow and to embrace diversity – culturally and geographically. Lisa was an example of the willingness to hold hands even around the globe, almost literally. Agreeing to work as a co-editor of the ESACT newsletter, jointly taking this job with Dr. Steve Oh, from Singapore, is a sign of this. If you reread parts of the September Newsletter you will find a lot of her enthusiasm and drive in the few words and ideas that go back to her.

Lisa was one of those young scientists in our field for whom I had great hopes – in many ways. We regret having lost her because we all

felt that there was so much more potential in her and that she had only started to transform a small part of it into reality.

Lisa left too early, we and even more her parents and siblings, pose questions that are difficult to answer. The photo shown here made her "famous" in Switzerland as it was used in a number of publications distributed by the Swiss National Science Foundation. It should remind us that doing science can be and should be fun. I believe this was one of the most important messages of this young scientist.

We will miss you Lisa.

Florian Wurm

A WORD FROM THE CHAIRMAN:

Dear ESACT Member,

I am please to announce the following news. Concerning the European funding programmes, there were in total about 15000 Expression of Interest (EoIs); with respect to part 1.1.1, Genomics and Biotech there were 1100. Recent contacts with the Commission indicated clearly that propositions such as ours **"Dedicated training activities in animal cell culture technology"** (ACTraining Network), a joint ESACT-ACTIP proposition would best fit into the Marie Curie Training Networks which will again be similar to the 6th Framework Programme. Such training networks which consist of at least 5 and at most 10 partners from industry, university or research institutions from at least three different countries will again exist in the future. The deadline of the first call will be the 25th of March 2003.

These are the following important evaluation criteria:-

- They have to be well structured with respect to the training.
- Propositions which are coordinated by a partner with expertise in management will have more chances to succeed than others.
- Coordination has to be done by a legal entity and, this is the reason why it cannot be done by ESACT nor by ACTIP (both organisations can at most support the gestation/establishment of a training network).

There are no pre-established targets in terms of scientific discipline or topic. The minimum qualification normally required is a degree that would allow the applicant to embark on a doctoral degree. I will contact the Commission, in order to verify, how engineers and students from specialized biotech schools which are not universities can also be trained. However, it is evident that training for technicians will not be financed via an EU programme.

In practice, a coordinating institution and a coordinator have to be found. The coordinator should be from a university institute active in the field of bio/chemical engineering or bioengineering. This person defines the final training programme and establishes the group of participants, which should be from different universities / research institutes and also from industry because the training programme deals with industrial animal cell technology.

ESACT's immediate activity will be to investigate whether there are training programmes for engineers, look for 10 partners to participate in a network, and second to forward the above information to all groups (institutes, industries) mentioned in the EoI.

The details on the organization, functioning, and the work programmes of the 6th Framework Programme is available since the 17th of December. Most of the proposed budget is planned for integrated « mega » projects (similar to clusters of previous EU programmes), and only 15% for small projects, but there is a push to increase this percentage to 30%.

Although the Commission likes these very large projects because this means less bureaucracy, the industrial community is very sceptical and hesitating to participate (for instance, only 4% of the EoIs were introduced by the industry). The management of such projects is very difficult and can only reasonably be performed by professional organizers and managers. Also for confidentiality reasons the industry prefers to participate only in small projects or not at all, in order to avoid the obligation to share the

scientific and technological advances with direct competitors.

18th ESACT-Meeting

The organization of the 18th ESACT-Meeting, which will be held in the nice city of Granada in Andalusia between the 11th and 14th May 2003, is well on the way (thanks to Francesc Godia from UAB/Barcelona). The submission deadline for the abstracts is over now, and the Organizing and ESACT Executive Committees will decide on the final scientific programme during a session in the beginning of February 2003. Five scientific sessions are planned for 18th ESACT-Meeting: **1. CELLULAR MECHANISMS** (Chairs: Jose Vicente Castell, Hospital La Fe, Valencia/E and Mike Butler, University of Manitoba/Canada), **2. CELL BASED THERAPIES -** Concepts and new approaches for mature and stem cell based therapies (Chairs: Martin Fussenegger, ETH Zürich/CH and Terry Papoutsakis, Northwestern University, Evanston/USA), **3. GENE BASED THERAPEUTICS -** Design, production and delivery of gene based therapeutics. This session is organized in cooperation with the European Society of Gene Therapy. (Chairs: Bernd Schröder, MainGen, Frankfurt am Main/D and Ernst Wagner, Ludwig-Maximilians-Universität, München/D), **4. TARGET DISCOVERY** (Chairs: Hansjörg Hauser, GBF, Braunschweig/D and Alain Bernard, Serono, Geneva/CH), **5. BIOPHARMACEUTICALS -** Improving the strategies for the production of biopharmaceuticals (Chairs: Carmen Vela, INGENASA, Madrid/E, and Christel Fenge, AstraZeneca, Stockholm/S). The following Keynote Speakers are confirmed: Prof. Mariano Barbacid. CNIO, National Cancer Center, Madrid/E, Prof. Richard Morimoto. Northwestern University, Evanston/USA, Prof. Axel Ulrich. Max-Plank Institut, Martinsried/D, Prof. Randal Kaufman. Howard Hughes Institute, Ann Arbor/USA, Prof. Herman Bujard. Zentrum für Molekulare Biologie, Heidelberg/D. More information can be found on the ESACT web site (www.esact.org).

As usual during each general meeting a general assembly will be organized. The agenda is not yet finalized, however, two points will be certainly addressed: first, the election of the new ESACT Executive Committee – you will be informed in due time, and, second, a proposal will be made to modify the constitution to put the associated members on the same level as the ordinary membership. For more details, please, take a look at the last issue of the ESACT Newsletters. Our friend Rod Smith from Xenova/Cambridge has already begun the organization of the 19th ESACT Meeting, which will be held in Harrogate/Yorkshire in springtime 2005.

Finally, the European Federation of Biotechnology (EFB) has sent out a call for the establishment of Branch Offices of the EFB. Learned societies, university institutes, national biotechnology associations, regional offices, professional management organizations or agencies specializing in facilitating information on or access to EC funding can apply. Applicants must have a qualified employee including necessary office facilities for tasks such as regional administration of EFB matters, dissemination of info, membership campaigns, regional meetings, and administrative support of one or more Sections or Task Groups to be specified. The deadline for submission is the 20th of January 2003. More information can be found on the web site of the EFB (www.efbweb.org) or directly from Boerge Diderichsen, President of EFB (bqd@novonordisk.com).

In closing, I wish all of you a happy and successful New Year.

Otto-Wilhelm Merten Crespières, 14.12.02

The UK Stem Cell Bank:
Summary for the European Society for Animal Cell Technology

The UK Stem Cell Bank at NIBSC: An Overview

Stem cell research offers enormous potential for major advances in clinical therapy against a wide range of important diseases, and the establishment of a UK Stem Cell Bank is an important step along the way to realizing this potential. The Cell Bank will offer a vital resource to support the advance of research in this exciting area. At the same time it will develop important safeguards, by ensuring that cell lines which could ultimately provide the basis for clinical treatment are appropriately characterized and also handled and stored

under conditions that are properly controlled. This will not only provide high quality starting materials to facilitate the development of stem cell therapy, but, in providing a centralized resource for researchers, should also reduce the use of surplus embryos for the development of stem cell lines by individual teams.

Cell banks are already in existence for many other types of cell line, but this MRC initiative, co-sponsored by BBSRC and with the full backing of the UK Government, will be the first Stem Cell Bank to be established worldwide, reflecting the UK's leading position in this exciting area of science.

The National Institute for Biological Standards and Control (NIBSC) is proud to have been selected as the host for the UK Stem Cell Bank. The Institute's mission is to assure the quality and safety of biological medicines through its research and development activities, and it has built up an international reputation, over many years, for its work on the safety and quality of vaccines and blood products. It also has direct experience of developing and archiving cell lines for clinical applications. Notably, NIBSC established and distributes the MRC-5 cell line that is now in routine use around the world for the production of adult and childhood vaccines. It is therefore a natural development for the Institute to take on the role of establishing and running the UK Stem Cell Bank, and helping to ensure as far as possible that stem cell therapy is developed in an appropriate framework of quality and safety.

The UK Stem Cell Bank will provide a repository for stem cells of all types, adult, foetal and embryonic, and will be developed to supply cell lines both for basic research and potential clinical applications. It will be operated in accordance with strict principles of governance laid down by a high level Steering Committee, chaired by Lord Patel, and will be managed on a day to day basis by dedicated and qualified staff trained to appropriate technical and quality standards. Close interaction and collaboration with the academic and clinical communities will be vital and joint project teams will be set up to assure the successful translation and establishment of each cell line into the bank. These teams will be directly supervised by a Management Group set up by NIBSC, including academic, clinical, regulatory and funding representatives. This group will be responsible for the management of the Bank, for publicizing its activities and for fostering interaction with the research and clinical communities.

Importantly, the establishment of the UK Stem Cell Bank should not be seen as an end in itself; instead, it represents a crucial element in the development of stem cell therapy as a whole. Ultimate success will benefit from a partnership approach, and NIBSC is committed to working closely with researchers, clinicians, funding bodies, industry and regulators, to bring this major opportunity for improving human health to fruition.

Aims of the UK Stem Cell Bank

The general aim of the UK Stem Cell Bank will be to create an independent and competent facility to produce, test and release seed stocks of existing and new stem cell lines derived from adult, foetal and embryonic human tissues. There will be two primary components in this work:

1) To provide banks of well-characterized stem cell lines for use in research in the UK and abroad. These will be established under well regulated, but non-GMP, conditions and made available in order to promote fundamental research.

2) To deliver banks of stem cell lines, prepared under GMP conditions, that could be used directly for production of human therapeutic materials.

Outputs of the UK Stem Cell Bank

In addition to the preparation and distribution of qualified and well characterized cell banks the UK Stem Cell Bank will also provide information on the cell lines in the bank and the technology used in their preparation and characterisation. The information will be made available through a dedicated bank web site, through annual reports from the bank, at open forum meetings and through collaborative training initiatives with the MRC and researchers in stem cell science.

The bank will be in a key position to contribute to the establishment of fundamental standards for the quality of stem cell products for therapy. The existing expertise available at

NIBSC in the safety and standardization of biological medicines will be utilized in collaboration with external experts to establish criteria and protocols to address safety and quality issues. The stability and reproducibility of stem cell lines during culture *in vitro* is critical to their use in both research and therapy. Thus, qualifying the *in vitro* functional reproducibility and stability of cultures will be an important element of the project. A highly detailed record of cell bank preparation, validation and testing procedures will be prepared for each cell bank and this information will be made available to users of the bank material.

Transparent operation of the UK Stem Cell Bank and close interactions with the user community will be critical to the success of the project. Thus the Management Group for the Bank will actively pursue a variety of means to communicate information on its activities, to enhance access to cultures and associated scientific data and to be responsive to the requirements and views of the user community and other interested parties.

The project will start officially in January 2003 and there will be a great deal of work to do to set up the bank. However, an NIBSC Stem Cell Bank Forum meeting will be held for interested parties at the earliest opportunity and accession procedures for cell lines for research will begin as soon as possible after the start date. NIBSC looks forward to close interactions with the stem cell community and welcomes views and comments from ESACT members.

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Large-scale Free Suspension Animal Cell Culture: Cell Fragility versus Media Homogeneity

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This short article reviews briefly the literature, especially from Birmingham and including work in bioreactors up to 8m^3 in volume, which is most relevant to the scale up of animal cell culture bioreactors. The work has shown that animal cells are considerably more robust with respect to mechanical stresses due to agitation than was considered originally. Hence, with the use of Pluronic F68, more vigorous aeration and agitation can be used on the large scale if (1) more intense cell culture conditions demand higher oxygen transfer rates; and (2) to overcome damaging spatial and temporal gradients especially of pH (and possibly of other parameters, e. g. dO$_2$) that tend to exist on the large scale though not on the small. With respect to pH, feeding into the regions of high specific energy dissipation close to impellers is particularly effective at reducing large local deviations from the desired control value, such deviations having been shown very recently to lead to significant levels of cell death.

Keywords:

cell culture, aeration, agitation, cell fragility, pH gradients

Introduction

This contribution is based mainly on work undertaken in the Centre for Biosystems Engineering at The University of Birmingham by the author and many others, which has attempted to elucidate the major determinants for successful large scale, suspension animal cell culture as a result of aeration and agitation. Work has been conducted at Birmingham since 1982 in bench scale bioreactors and, relatively recently, this work has been extended up to the industrial scale (8 m3 working volume) as part of a DTI/BBSRC Biochemical Engineering LINK Project involving collaboration with GlaxoWellcome (at the time the project was completed). Other work is also reviewed as appropriate.

Small scale studies related to mechanical stresses

The work at Birmingham has shown that even at agitation intensities of 200 W m-3, which are almost as high as those found in bacterial fermentations (~ 1,000 W m-3), a wide range of cell lines (e.g., TB/C3 hybridomas, Sf 9 insect cells, NS1 myelomas, etc.) grew quite satisfactorily under unsparged conditions (Kioukia et al., 1992, 1995a, 1996; Oh et al.,

1989, 1992). These studies also confirmed that while bubble sparging could be lethally damaging to the cells, the use of Pluronic F68, as first shown by Kilburn and Webb (1968), prevented it (Handa-Corrigan et al., 1989; Kioukia et al., 1992, 1995a, 1996; Oh et al., 1992).

Measurement of cell strength by micromanipulation (Zhang et al., 1993) supported the conclusion that cells can withstand significantly more severe agitation conditions than was initially thought (Kioukia et al., 1995b). Modelling of the stresses associated with bursting bubbles also showed the huge local specific energy dissipation rates (Boulton-Stone & Blake, 1993) and stresses experienced by cells if attached to them. However, such attachment is prevented by Pluronic F68, hence its effectiveness (Chalmers & Bavarian, 1991).

Large scale studies; inhomogeneity and mass transfer

The bioreactors at Wellcome were designed more than 25 years ago when concern for 'shear' damage to cells was a major factor in determining the agitation conditions. As a result, they operate with very low agitation speeds (1.0 rev. s^{-1} max) using a small Rushton turbine (~ 1/5 of the vessel diameter) giving a maximum specific power input of ~ 10 W m^{-3}, typically two orders of magnitude less than those found in bacterial fermentations. Aeration rates, because of low oxygen demands by the cells, were also made very small (0.005 vvm). Experimental analysis of the physical characteristics of actual bioreactors up to 8 m^3 were undertaken, alongside geometrically-scaled down transparent versions for flow visualisation (Langheinrich et al., 1995, 1996, 1999, 2001; Nienow et al., 1996a).

Biological characteristics (oxygen uptake rate, cell growth, viability and production) were also determined (Nienow et al, 1996a). Though specific oxygen uptake rates with different cell lines varied by an order of magnitude (Nienow et al, 1996a,b), all the lines studied grew well, with a CHO 320 line (which was studied in most detail) doing so over a dO_2 range between 20% and 80% of saturation with equal interferon-γ production over this range. However, all cell lines were sensitive to pH, which is nominally controlled to ± 0.2 units by a pH probe placed close to the impeller, leading to alkali or CO_2 addition as appropriate (Langheinrich and Nienow, 1999).

The interaction of the sparged air with the agitator is negligible, so that the impeller is completely flooded. However, the flooding phenomenon is very different from that in microbial fermenters. The difference arises because of the very low air flow and energy dissipation rates, so that, whilst the bubbles rise essentially vertically up the middle of the bioreactor dragging some fluid with them, the impeller still pumps some fluid radially. In spite of the difference, literature correlations for the oxygen transfer coefficient, k_La, (Langheinrich et al, 1996, 2002; Lavery & Nienow, 1987), obtained at much more vigorous operating conditions, apply moderately well though clearly the absolute values are very low. An analysis of characteristic times suggests that more intensive culture conditions or the use of cell lines with a higher specific oxygen demand could lead to oxygen mass transfer limitations on the large scale, although dO_2 gradients should be small (Langheinrich et al, 1995). However, since k_La is essentially independent of agitator type, it can only be enhanced by using higher air flow or agitator specific power inputs (Langheinrich et al, 2002).

Mixing characteristics were also studied and again, even though the air dispersion was extremely different, literature correlations for mixing time, **Θ_m**, (Langheinrich et al, 1995, 1998; Nienow, 1997) apply quite well. The results and correlations clearly show that, under these very mild agitation conditions, mixing is very slow, especially at aspect ratios, AR > 1 on the large scale (Θ_m at equal W/kg increases with (scale) $^{2/3}$). In this case, Θ_m could be reduced by about 20%, without increasing agitation intensity, by retrofitting the current small Rushton turbine with a larger diameter, low power number agitator. Addition into the impeller region, especially at AR > 1, is also very effective (see below).

Because of this poor mixing, pH probes and flow visualisation (recorded on video) showed that the method of pH control by 3 M Na_2CO_3 addition on to the top surface was very unsatisfactory. The upper region of the bioreactor is particularly badly mixed and this mode of addition gives rise to significantly

higher levels of pH just below the liquid surface (Nienow et al, 1996a; Langheinrich and Nienow, 1999). However, feeding into the impeller can essentially eliminate such excursions in pH (Langheinrich and Nienow, 1999).

Very recently, Osman et al. (2002) undertook a scale-down study to mimic the above observations. GS-NSO cells and media containing Pluronic were circulated between two sparged bench scale bioreactors, one held at pH 7.3 and one at pH 8 or 9. This work clearly confirmed that when cells experience regular cyclic excursions through regions of high pH, there is a significant increase in cell death (up to about 30%).

Conclusions

It is concluded that locally high levels of pH are potentially more damaging in large-scale, free suspension cell cultivation than stresses due to agitation and sparged aeration (provided Pluronic F68 is used). Such excursions can be essentially eliminated by feeding alkali into the impeller region. If enhanced oxygen transfer rates are required, agitation and/or aeration intensities have to be enhanced. It is very probable that such enhancements can be safely utilised, given the knowledge that we now have about cell strength and the role of Pluronic.

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Summary of the 1st Microcarrier Workshop on microcarriers for large scale cell culture. Rome/I, 3rd-4th October 2002.

Time and location: The workshop was held at the Hotel Antonella in Pomezia near Rome on the 4th of October. A get together dinner was organised in the evening prior the meeting.
Statistics: 29 people attended, from industry leaders in microcarrier technology potential users and academics. People from 19 different companies, institutions out of 11 different countries came.
Feedback: The feedback was overall very positive. All of the attendees wanted to have a follow up meeting in 2 years time.
Topics: Scientific contributions from vaccine industry, gene therapy, rec. protein production, medium optimization, fluidized bed technology, cell mass detection on microcarriers, history, microcarrier applications, strategy for retro virus production.

Summary of the contributions

Microcarrier technology, from invention to large scale production.
Dr. T. van der Velden de Groot (RIVM, The Netherlands) presented an historical overview on the development of Cytodex I and Cytodex III microcarriers, which were then commercialized by Pharmacia (now Amersham Biosciences) and became the basis for the large scale production of viral vaccines using Vero cells at the Institut Mérieux, now Aventis Pasteur. By coincidence exactly 35 years prior to this workshop the revolutionary publication from van Wezel in Nature came out (7/10/67). Several years were needed to optimise the surface of microcarriers to make them suitable for industrial processes. The optimal surface charge of such carriers was established to be 1.8 meq/g carrier for primary cells. A production process of monovalent IPV in a 700 litre reactor was discussed. Pictures of RSV infection (after 48, 72, 96 and 120 hours post infection) on fully colonised Cytodex 1 with Vero cells were shown. A very interesting observation important for the determination of optimal time point for virus harvest was that the highest OUR (oxygen uptake rate) appeared one day before the highest virus titer was obtained. This observation allows a very precise virus harvest at a time at which the highest virus titer with low cell lysis is obtained. Too a late harvest would lead to cell lysis followed by increased problems during downstream processing.

Experiences in large scale microcarrier fermentation.
Dr. M. Tanner (Werthenstein Chemie AG, Switzerland) presented a large scale production process of rAdV on 293 cells grown on Cytodex III carriers. The scale up was performed starting from 1 T-75, via 1 T-500, 3 T-500, 1 CF-10, 4 CF-10, 4 CF-40, 12 CF-40, and a 500 l bioreactor to the 2000 l production scale reactor. The optimal scale up protocol would be the replacement of the 4 CF-40 and 12 CF-40 steps with 20 l and 100 l bioreactors. However, due to equipment constraints this was not possible. In order to keep the highest hygiene standard, they use only large scale peristaltic pumps for all transfers to be done. The carriers are swollen in PBS/Tween 80 at 4°C or 20°C for 3 hours, followed by a washing step for eliminating the Tween and a transfer to the reactor. Then the carriers are sterilized in situ together with the reactor (121°C, 50 min). Before inoculation of the culture, the carriers are washed with PBS and medium and incubated for equilibration. They use 2-4 g carriers per l and inoculate 8-20 cells/carriers. The optimal conditions are a pH of 7.2-7.4 and a pO_2 of about 40%. They work in a perfusion mode in order to maintain a relatively low lactate concentration. The carriers are retained by using a spinfilter, the maximal perfusion rate is one working volume per two hours. As critical scale-up parameter a constant power input was used. The agitation speed in the 2000 l reactor is 15-25. This speed is normally increased by a factor of 3 for detaching the cells during trypsinisation. The medium containing 10% FCS is diluted to a FCS content of 1%, trypsin is added, and as soon as 90% of the cells are detached, serum is added to inactivate the trypsin activity and the cells are transferred to the next reactor size,

which contains already equilibrated new Cytodex III carriers. The minimum cell viability should be 80% in order to get a short lag phase.

Production of DISC-HSV using microcarrier culture.

Dr. R. Smith (Xenova, U.K.) presented the use of Cytodex I carriers (5 g/l) for the production of DISC-Herpes Simplex Virus 2. Using 5 g/l of carriers a batch process can still be used, however, above this quantity a perfusion process has to be employed. The whole process has been optimised with respect to pH conditions for cell attachment, cell growth, and virus production, passaging regime etc. For passaging an internal sieve is used for separating the detached cells from the carriers. The cell detachment is done by using a hypotonic saline solution. In order to improve cell detachment, the agitation is increased during the detachment process. For virus production, the complementation cells are infected with virus. For virus harvest, the cells are disrupted by low pressure shear, followed by a clarification and further purification steps. The scale up is done from a 5 l to a 20 l, a 100 l and finally to a 500 l reactor. The critical scale-up parameter was the tip speed of the impeller. It takes about 35 days to get from a T-flask to the production scale reactor. As no perfusion is done for the moment medium changes are done after sedimentation of the carriers.

Use of microcarrier technology for the production of rabies vaccines in BHK-21 and Vero cells.

Dr. H. Kallel (Institut Pasteur de Tunis, Tunesia) presented the production of veterinary and human rabies vaccines using BHK-21 and Vero cells, respectively. For both processes 3 g/l of Cytodex III and Cytodex I carriers are used allowing the production of $3x10^6$ cells/ml or $4-5x10^6$ cells/ml in case of BHK-21 and Vero cells, respectively. The cultures are done in a perfusion mode in order to maintain the residual lactate concentration at relatively low levels (15 mM for the BHK-21 cultures, 5 mM for the Vero cultures). The BHK-21 process was scaled up to 20 l providing $2x10^8$ FFU/ml or 40000 doses per run (in 30 l of virus suspension) = 2 IU/dose (better than the international standard). The Vero process (not scaled for the moment) provides a titer of $7x10^6$ FFU/ml in a perfusion mode or $2x10^7$ ffu/ml in a recirculation mode. The concentrated and inactivated harvest contains 10 IU/ml (- the WHO demands a minimum of 2.5 IU/ml). They are working on the serum-free process, because the actual processes still based on the use of serum.

Microcarrier applications for R&D and manufacturing processes.

Dr. M. Reiter (Baxter BioSciences, Austria) presented 6 different carrier applications which are used in house by Baxter or for commercial productions:

- Vero, protein-free, Cytodex III – viral antigens for research. They developed their own protein free cultured Vero cells. The span for production is from passage number 133 to 145.
- Lymphoblastoid cell line, protein-free, Cytopore. Human IgG1 for research purposes. Purpose of using macroporous microcarrier is to simplify the change from serum to protein free conditions.
- Vero, protein-free, Cytodex III – production of influenza virus (actual scale: 1300 l, planned up to 10000 l). Validation runs parameter: Oxygen uptake, residual Glucose.
- Transfected Vero cells, protein-free, Cytopore 2 – HAV-vaccine, 100 l perfusion mode, 2462 l -> 57.5 M HAV-antigen, $3x10^6$ doses. 100 litre fermenter running for 200 days
- Vero, protein-free, Cytodex III – production of a Ross River Virus (epidemic polyarthritis) candidate vaccine (in Australia 8000 cases/year).
- Vero, protein-free, Cytodex III – production of a small pox vaccine (Vaccinia virus).

For all Vero processes: biomass production is in a perfusion mode, virus production in a batch/fed-batch mode.

Growth of recombinant BHK and CHO cells in microcarrier culture.

Dr. I. Molgaard-Knudsen (Novo Nordisk, Denmark) presented 4 microcarrier applications for the production of recombinant proteins:

- rBHK, serum-containing medium, Cytodex III (inoculation: 2 cells/carrier, scale-up via carrier-carrier migration). 500 liter fermenter running 35 days.

- rBHK, animal substance-free medium, Cytodex I 5g/l (good cell adhesion and cell growth, critical parameters: inoculation cell density and pH (< 7.2). The presence of Pluronic F68 is necessary in order to get a good cell adhesion. The scale-up is very difficult)
- rCHO, serum-free process, Cytopore1 (because no good perfusion system at large scale available for suspension cells. Inoculation cell density: 20000 c/cm^2, carrier-carrier migration for scale-up). Carrier are fully colonised after 6 days. 10 to 20% free cells in culture.
- Rec. BHK. Serum free BHK medium, Cytopore 1 as carrier in a 10 l fermenter and 50 days of culture. Cell count is a problem. Use of the Aber Biomass Monitor for following the cell growth on/in Cytopore carriers – rather good and precise control system.

Influence of cell culture media on the adherence of animal cells on microcarriers.
Dr. K. Landauer (IAM, Austria) presented factors influencing cell adhesion to and cell detachment from Cytoline 1 carriers, which are of interest for performing fluidized bed reactor in a perfusion mode. In this project the influence of a number of media components (bivalent cations, different iron concentrations, hydrocolloides and other biopolymers of non-animal origin) on the attachment and detachment process on microcarriers has been tested with special focus on large scale usage (price, availability, quality etc.). A three step test method was carried out. 1. a so called vibrax system to be able to test many substances in T-flasks. 2. The substances turning out to increase adherence are then tested on microcarriers in small scale. 3. The best substances and concentration will be tested in a lab scale fermentation. The effect of different ferric citrate concentrations and dextran derivatives on the adhesion of cells in T-flasks and on macroporous microcarriers was shown. Tests were carried out with different recombinant Chinese Hamster Ovary (CHO) cell lines on Cytoline 1$^{(TM)}$ (Amersham Biosciences) microcarriers in protein-free medium. First results showed that certain carboxymethyl dextrans and low ferric citrate concentrations improve attachment. In addition the cells are more shear resistant resulting in higher cell densities on the microcarriers.

Use of the fluidized bed system CYTOPILOT MINI for the production of retroviral vectors for gene therapy. Growth of PG13 cells on Cytoline 1 carriers.
Finally, as last speaker, Prof. Otto-Wilhelm Merten, Généthon, France presented the use of the fluidized bed system Cytopilot Mini for the production of retroviral vectors for gene therapy using PG13-cells grown on Cytoline1 carriers. The production of high titer retroviral vector preparations is an important prerequisite for their successful application for gene therapy purposes. They have evaluated the fluidized bed reactor (CYTOPILOT MINI) for the production of retroviral vectors using PG13 producer cells. Consistent cell growth was obtained when 3x10^8 cells were inoculated on 150 ml of Cytoline I carriers. A culture of a duration of about 336 hours needed about 30 l of culture medium and could produce a total vector quantity of 1.33x10^{10} infectious particles (average titer : 4.3x10^5 ip/ml) at an estimated total viable cell number of 0.43x10^{10} cells. The comparison to a standard process based on the use of a CellCube module 25 system and normally used at Généthon leads to the conclusion that both systems provide a rather similar vector production. A culture of duration of 157.5 hours, produced in total about 1.14x10^{10} infectious particles with an average titer of 6.1x10^5 ip/ml. The total viable cell number was estimated to be 2.2x10^{10} cells. However, this comparison indicated also, that with respect to cell numbers, the use of 600 to 750 ml of Cytoline I carriers would lead to equivalent cell numbers and cumulative vector titers as obtained in a CellCube 25 system.
Although the biological parameters were rather similar for both culture systems, the fluidized bed reactor system provides at least two advantages when compared the fixed bed reactor system (CellCube). Whereas the parallel plates of the CellCube will become completely covered by the cells leading eventually to double/triple- (as for PG13 cells) or multi- (as for TeFly cells) layers with a physiologically heterogeneous cell population, the situation is quite different for the fluidized bed reactor. Here, the cells start to fill the large caverns of the carriers (which are very porous), as shown by confocal microscopy, however, shear forces as well as convection through the channels which cross the beads seem to avoid the complete filling up of the space and the

formation of tissue like cell masses with all its associated inconveniences. Thus long term steady state cultures are possible which should be avoided in fixed bed reactor systems (CellCube, NBS basket reactor) for cells whose growth is not or only partially contact – inhibited. The second advantage concerns scale-up issues. Whereas the largest size of the CellCube provides only approximately 340000 cm^2 (= module 400 which is equivalent to 16 25-modules), the CYTOPILOT fluidized bed reactor has successfully scaled to much larger sizes.

O.-W. Merten (Généthon, Evry/F) & G. Blüml (Amersham Biosciences, Vienna/A) 9.12.02

Conference Report - 224th ACS Meeting

The following synopsis is focused particularly on the subject of BIOSEPARATION, in the context of biopharmaceutical manufacturing. The content is based on the collation of selected talks and questions-and-answers provided by various academics, industry partners, consultants, critics and students during the sessions.

Feeling the Squeeze

The rapid advancement and expansive growth of biosciences has lead to an increasing number of new biological therapeutics entering the development pipeline. Industrial analysts foresee the need to enlarge manufacturing capacity by at least 4-fold to match the exuberant number of candidates progressing to the clinical trial phase and the market (for a fortunate few). This capacity demand can be tackled by a number of approaches. The straightforward resolution is to build more manufacturing facilities, which often involve a substantial amount of capital investment. Other measures being employed include, the ‘stretching-out’ of existing capacity by maximizing the utilization of plant equipment and by multiplexing production campaigns. Amgen is exploring the option of cycling smaller purification columns operating in batch mode, an operation termed as ‘batchtinuous’ by the presenter, **Peter Walter**. The rationale for such an operation were: (i) reduction in capital investment due to the use of smaller equipment; (ii) and column cycling that significantly lowered the present value cost of the process, based on their pilot studies conducted in Thousand Oaks, CA.

Capturing Opportunities

Another important fact quoted vehemently by a number of industry speakers was that the majority of these new biologics in the early and final stages of development was none other than the monoclonal antibody-based therapeutics. **John Curling**, a biotechnology consultant based in Sweden, said that around 1000 new monoclonal antibody-based products could be entering development in the next 10 years and 70 new approvals should be anticipated by the year 2010. This anticipated expansion in product output implies that all the requirements for the manufacturing of such biologics must be multiplied correspondingly. In particular, the supply of Protein A required for capturing monoclonal antibodies during downstream processing is probably insufficient to meet such increasing demand. Certain companies which envisaged the critical shortage of Protein A have also ventured into the development of synthetic adsorbents (*e.g.* MAbsorbent A2P) equivalent to Protein A’s functionality. Such synthetic adsorbents are notably cheaper (approximately 20% of the cost of Protein A) and possess considerably higher binding capacities (41-47 mg/ml).

Continuous Solutions

Ideally, if the processes can be operated on a (truly) continuous mode, a further extension in manufacturing capacity should be attainable. However, fully continuous bioprocessing in downstream separation is yet a common practice in commercial production environment, due to various technological as well as operational constraints (*e.g.* regulatory issues). In more recent years, the persistent pursuit of continuous bioprocessing by some groups has lead to innovations that transformed two separate technologies, previously used in other kinds of processes, to a platform suitable for bioseparation: (i) continuous annular chromatography (CAC); (ii) and simulated moving bed (SMB).

The idea of CAC was suggested and discussed by **A.P. Martin** in 1949. Much later, the theoretical feasibility of CAC was demonstrated by J.C. Giddings in 1962, and D. Dinelli and coworkers built a rotating gas chromatograph consisting of 100 columns on the same year. However, the first true CAC

system was developed and constructed by J.B. Fox and coworkers in 1969. Their creation was already very much like the modern CAC units. In the late 90's, Prior Engineering (Austria) began commercial manufacturing of CAC equipment, first for the separation of valuable metals and more recently for a number of applications in bioseparation.

Alois Jungbauer (presenter from University of Agricultural Science, Vienna) and coworkers reported that higher productivity could be achieved using the CAC compared to conventional packed bed batch operation. They demonstrated the running conditions stability of CAC packed with Superdex 200 prep grade resin using human serum albumin as the model protein solution, and found that the eluted peak started to shift only after 8 days (>190 hours) of continuous operation. In addition, they revealed that the resolution was independent of the rotation rate whenever a constant loading factor was ensured; whereas at a constant feed rate, the resolution could be improved slightly with increasing angular velocity. At present, the CAC system seems to be capable of performing most modes of chromatography (*i.e.* gel filtration, ion exchange and affinity), however the separation capability of ion exchange chromatography is restricted since elution can be performed in the step gradient mode only (*i.e.* linear gradient elution is not possible). More importantly, its utility in industry is still uncertain, as issues relating to process validation are mostly unsettled.

The SMB technology was developed in the early 1960's under the generic name of Sorbex. Initially, SMB were primarily applied in the petrochemical industry (for large scale separation of hydrocarbons) and in the 1970's, it found its way into the food-related manufacturing industry, involving separation of sugars. In the 1990's, it was another major industry, the pharmaceutical manufacturers, that has exploited the SMB technology to separate chiral compounds or enantiomers. More recently, manufacturers of large scale SMB system are turning their attentions towards protein-based product separation derived from the biotechnology industry. **Grant Epsie** (EM industries Inc., NJ) and **M. Schulte** (Merck KGaA) presented their work on the use of size exclusion chromatography-SMB to separate smaller proteins within the fractionation range of the gel (Fractogel BioSEC) and the purification of a large protein from its aggregates. In addition, the applicability of two different lab-scale SMB systems (Licosep of France and WellChrom of Germany) was discussed briefly. Aside from one particularly strong advantage of this technology (*i.e.* capable of continuous operation), there is a number of drawbacks which have prevented it from making more inroads into many other separation processes. A key disadvantage of the present SMB technology is its limited separation scope, involving only the fractionation of binary mixture. In theory, N-1 units of SMB systems are necessary to separate N compounds. However, some 'die-hard' proponents of SMB technology have been working towards extending the 'walls' of separation capability. For instance, a group (**Luuk A.M. van der Wielen *et al*.**, Kluyver Lab. for Biotechnol., Netherlands) has reported their development of methodologies to perform ion exchange chromatography by step gradient on a simplified SMB framework (4-section unit). Until the next big leap is made, the applicability of SMB in bioseparation has been less than significant.

Location: Boston City, MA
Date: 18th to 22nd August 2002
Prepared by: Dr. Goh Lin-Tang, Bioprocessing Technology Centre, National University of Singapore btcglt@nus.edu.sg

Joke Corner

Evolution?

The story is told of a gorilla which escaped from the zoo. They searched everywhere for it. Finally, they found the gorilla sitting in the city library pouring over a copy of the Bible and a copy of Darwin's Origin of Species. When asked what he was doing, the gorilla replied, "I am trying to figure out whether I am my brother's keeper or whether I am my keeper's brother."

Bosses!

A scientist, a technician and a vice-president of a biotech company are on their way to lunch and suddenly stumble across an antique oil lamp. They rub it and a Genie comes out. The Genie says, "I usually only grant three wishes, so I'll give each one of you just one. "Me first! Me first!" says the scientist "I want to be in the Bahamas, without a care in the world". Poof, she's gone!
In astonishment, "Me next! Me next!" says the technician. "I want to in Hawaii relaxing on the beach with the love of my life. Poof! He's gone.

"You're next," the Genie says to the vice president. The vice president says, "I want those two back in the office after lunch."

Moral of story: Always let your Boss have the first say…

TRADITIONAL CAPITALISM

You have two cows. You sell one and buy a bull. Your herd multiplies and the economy grows. You sell them and retire on the income.

AN AMERICAN CORPORATION

You have two cows. You sell one and force the other to produce the milk of four cows. You are surprised when the cow drops dead.

A FRENCH CORPORATION

You have two cows. You go on strike because you want three cows.

JAPANESE CORPORATION

You have two cows. You redesign them so they are one-tenth the size of an ordinary cow and produce twenty times the milk. You then create clever cow cartoon images called 'Cowkimon' and market them World-Wide.

A GERMAN CORPORATION

You have two cows. You re-engineer them so they live for 100 years, eat once a month, and milk themselves.

A BRITISH CORPORATION

You have two cows. Both are mad.

AN ITALIAN CORPORATION

You have two cows, but you don't know where they are. You break for lunch.

A SWISS CORPORATION

You have 5,000 cows and none of which belong to you. You charge others for storing them.

A CHINESE CORPORATION

You have two cows. You have 300 people milking them. You claim full employment and high bovine productivity. You have the newsman who reported on the numbers arrested.

AN INDIAN CORPORATION

You have two cows. You worship them.

NEW MEMBERS

ESACT welcomes the following new members.

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With Best Wishes for 2003 **Bryan Griffiths**

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