

# THE ESACT NEWSLETTER

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## **Editorial**

Hello Readers! It's coming up to a year since Lisa & I enthusiastically volunteered for these positions as editors. Since then, we have strived to increase the involvement of ESACT members in the evolving newsletter. So far, we have encountered sparks of enthusiasm and participation, and also much silence.

Perhaps it's a silence of neutrality or of glad acceptance, but we hope not of diffidence. Either way, we encourage you to share your comments and suggestions: email [Steve.Oh@esact.org](mailto:Steve.Oh@esact.org). For starters, ideas for improving the newsletter proposed by committee members are summarized in the pages within. We'd like to hear your feedback too, friends!

I had also hoped to bring to you an old wizened article from Prof. Ray Spier, but unfortunately I've lost my precious ESACT '89 Proceedings. If anyone has a 'blast from the past', please feel free to contribute.

In this packed issue, we bring you a Word from the Chairman concerning some exciting news about membership developments and an initiative to train more experts in animal cell technology via an *EoI* (Find out what that is within). We are rethinking cell culture, giving you stem cell updates, asking for proposals for a legal framework for tissue engineered products, reporting 2 ACS conference meetings, giving a "Hot!" invitation and throwing in some mirth at the joke corner. **ENJOY!**

A satisfied Chief Editor (-\_-)

Steve Oh. Singapore, 5<sup>th</sup> Sept. '02

**A WORD FROM THE CHAIRMAN:**

Dear ESACT Member,

We are now in the middle of the holiday time and I hope that most of you will have had your holidays when you read the present ESACT Newsletter. For me, the summer holidays mean a break in the routine and a time for reflection and summarizing.

What was achieved by ESACT during the last year? The organization of the next meeting, the 18<sup>th</sup> ESACT-Meeting, which will be held in the nice city of Granada in Andalucia in May 2003, is well on the way (thanks to Francesc Godia from UAB/Barcelona) In the meantime, everyone should have received the second announcement concerning this meeting (which can also be found on [www.esact.org](http://www.esact.org)). The future is coming rapidly, and as soon as the 18<sup>th</sup> ESACT Meeting is over, the "organizer's stress" will shift to England. Rod Smith from Xenova/Cambridge has begun the organization of the 19<sup>th</sup> ESACT Meeting, which will be held in Harrogate/Yorkshire in spring 2005. The view up to 2007 is still foggy, signifying that things are open with respect to the meeting place and the meeting organizer.

The way to the other side of the earth brings us to Japan, and there you can meet our Japanese friends from JAACT, who will celebrate the 15<sup>th</sup> Annual and International Meeting of JAACT (the JAACT 2002 FUCHU Meeting), which will be organized by Kazumi Yagasaki. Everybody is kindly invited. For more information, please take a look at the meeting website [www.tuat.ac.jp/~jaact02/](http://www.tuat.ac.jp/~jaact02/)

As for meetings past, the last Cell Culture Engineering Meeting, which was organized by Michael Bethenbaugh and John Aunins in Snowmass/CO at the beginning of April this year, was a success with many excellent oral and poster presentations. Selected manuscripts will be published in a special issue of *Biotechnol. Prog.*; for a report on the meeting, see later. During this meeting, there was ample time for discussion with the organizers of the Cell Culture Engineering Conferences on future interactions/contacts between them and ESACT. It was decided to strengthen contacts and to coordinate common activities. At least partially in this context, the committee has started to discuss the status of associated members, because it seems anachronistic and also unjust to maintain different membership static for Europeans and Non-Europeans. A proposition for modifying the membership status of associated members will be put forth for the next General Assembly. However, as this matter should be openly discussed from now until Granada, I invite everybody to contact the members of the Executive Committee and express his/her opinion. For more details, please, take a look at the article "**PROPOSITION FOR THE EVOLUTION OF ASSOCIATED INTO ORDINARY MEMBERSHIP STATUS**".

What else? The EU is still working on the final formulation of the 6<sup>th</sup> Framework Programme (FP6) and everybody hopes that it will be out soon. In contrast to the previous programme, FP 6 is a change in strategy and focuses on realising the European Research Area, with a strengthening of EU expertise in certain thematic areas. There will be no more room for relatively small projects and

dozens of different topics. Of importance to ESACT members is priority 1: Life sciences, genomics and biotechnology for health. Emphasis will be on health applications. The total budget is 2.2 billion Euros. To attain the objectives, two new instruments, 'Networks of Excellence' and 'Integrated Projects', will be in effect along with some of the modalities that existed previously (the new version of accompanying measures, and some support for smaller, targeted R&D projects). Specific measures such as co-operative research and collective research projects have been created for SMEs. 'Integrated Projects' represent very large projects (in the order of 10-20 mEuros), with a targeted objective. They will be autonomous and flexible, meaning that partners can be added or eliminated during their execution. 'Networks of Excellence' are much broader projects intended to run for 5 years. The partners will include SMEs and industry. As a remark, 15% of the budget of priority 1 will be spent to support SMEs.

Most of you are aware that the 6<sup>th</sup> Framework Programme has started with a call for 'Expression of Interest' (or EoI) for two domains: Networks of Excellence and Integrated Projects. About 15,500 EoI were received at the deadline, 7<sup>th</sup> of June 2002. The commission will use this input for the formulation of the first call for proposals for the 6<sup>th</sup> Framework Programme. As it seems that there is a lack of qualified personnel of all categories (technician, engineer, PhDs) in the field of animal cell technology (in particular, in large scale manufacture/processing), ESACT and ACTIP have decided to introduce an EoI for a network of excellence, entitled "Dedicated training activities in animal cell culture technology"

(acronym: ACTraining Network). Although this field is not really in the core of the actual call, the intention was to inform the commission of the needs of the European Biotech Industry active in the field of large scale animal cell technology and to get a piece of the pie for training activities, probably in the frame of an 'Integrated Project'. The abstract of this EoI can be found in this edition of the ESACT Newsletter, and the whole text of the EoI can be found on the ESACT website ([www.esact.org](http://www.esact.org)).

This ESACT-ACTIP joint initiative should also be seen in the light of the future capacity of the biopharmaceutical industry. This industry has experienced healthy growth during the past few years, with drug sales from recombinant proteins reaching an estimated \$19 billion in 2000, a 20% growth rate as compared with 1999. However, with more than 900 biologics in development (preclinical studies to Phase III clinical trials), the best has yet to come, indicating that actual production capacities are largely insufficient for the projected production boom. Some analysts predict that 100 monoclonal antibody products, of which several have to be produced at a scale of tens to hundreds of kg, could be approved for market by 2010. To put things into perspective, the world's largest mammalian cell culture facility (Genentech, Vacaville/CA) has an approximate capacity of 96000 l. It is estimated that this total capacity could be fully used by as few as 6 successfully commercialized monoclonal antibody products. Thus, in 2010, 16 new Vacaville-type production plants will be needed if all 100 monoclonal antibody products pass clinical trials. These predictions, which have been published in more detail by Lias R. & Fogerty S. in the

June issue of Pharmaceutical Technology Europe (2002, pp. 31-34), underline the need for bioindustry to carefully plan its own investment in production plants or to manage outsourcing to contract manufacturers well ahead of time, but also signify the increasing need for trained people in the field of large scale bioprocessing.

In closing, I wish to those who will be going on vacation, nice holidays with a lot of sun and less rain as presently in Europe, and to those who are already back from vacation, a good and successful start in the autumn season.

Otto-Wilhelm Merten                      Evry, 9.8.02

**“PROPOSITION FOR THE EVOLUTION OF ASSOCIATED INTO ORDINARY MEMBERSHIP STATUS”.**

During the last General Assembly of ESACT held in Tylösand last year, there was a short discussion on the question of whether ESACT should transform associated membership status (held by all non-European members of ESACT) into ordinary membership status (held by all European members who live and work in Europe). This point was raised because associated members pay higher membership fees than ordinary members, yet they have practically no possibility of influencing the business of ESACT or even taking part in the General Assembly. In principle, this is anachronistic and also peculiar because most of the other European professional societies no longer make this distinction.

Our world, which was seen as a disc without defined limits or frontiers several

centuries ago (at least in Christian Europe), has in the meantime become a world village due to a process called ‘globalization’. ESACT should not swim against the stream. The question here is not whether globalization is progress or a set-back. Globalization is now a fact of life, and ESACT should become more open to the rest of the world. This will not only benefit our society, but will also benefit our colleagues and collaborators around the world.

Let me remind you that there is no society for animal cell technology in the USA (or the Americas, in general) and presently there are no concrete plans to form one within the USA. This signifies that the opening of ESACT to Non-Europeans will encourage those which are active in animal cell technology to join ESACT and to participate in ESACT-sponsored meetings, but also to profit from various ESACT-supported services and activities. The proposal for a single membership status for everyone, independent of his/her residence or working place, will be a gesture of welcome to our Non-European friends and colleagues. The offering of equal membership status to our overseas members will have a second important advantage/impact. If the next General Assembly accepts the proposition to transform associated membership into ordinary membership, it is rather improbable that our American friends will feel the need to create an American equivalent of ESACT.

On the contrary, as we move along the lines of “equal membership fee, equal rights, equal partnership”, a possible further development in the mid- or long-term might be the creation of an International Society for Animal Cell

Technology (ISACT). ESACT will certainly play a central role in this development either by becoming the ISACT or by being the driving force for the creation of ISACT. This potential future development is currently under evaluation within the Executive Committee and will certainly be a point for discussion at the next General Assembly.

Thus, the ESACT Executive Committee will prepare for the discussion and eventual decision on the transformation of associated membership at the General Assembly at our next ESACT meeting in Granada in 2003. As for ISACT, the Executive Committee feels it is time to start the discussion but sees no need to rush.

In closing, I invite every ESACT member to contribute arguments, positive or negative, to these propositions, in order to get a real discussion on this matter going. As you know, I have recently sent a letter to the members dealing essentially with the same issue. The replies received so far were more than positive. In any case, please, feel free to contact me or any other member of the Executive Committee for more information or clarification. We are committed to creating, with you and for you, the members of ESACT, a better future for our society.

Otto-Wilhelm Merten Evry, 8.8.02

**Abstract of the EoI on « Dedicated training activities in animal cell culture technology » (ACTraining Network):**

With this expression of interest ACTIP, the Animal Cell Technology Industrial Platform, representing Europe's industry using animal cell culture technologies, together with ESACT, the European

Society for Animal Cell Technology, representing individual scientists working in the field, would like to draw attention to the current and future shortage of qualified personnel at all levels (technician, engineer, PhD) in animal cell culture technologies. This shortage, which is expected to become worse with increasing activities in biotechnology, genomics, proteomics, metabolomics and toxicogenomics, could severely hamper Europe's scientific and industrial competitiveness and should be addressed by a dedicated "training through research" programme relevant for the biotech industry active in Animal Cell Technology (including production of biopharmaceuticals, cell-biology and screening methods). Such a training programme could be executed in both academia and industry and would address all levels (from technicians to PhD students).

Because of the specialised nature of the profession and the shortage of national facilities in this field in most EU member states, a co-ordinated European effort is the only realistic option. Therefore, ESACT and ACTIP urge the European Commission to create opportunities in Framework Programme 6 to support **dedicated "training through research" activities in animal cell culture technology** to adequately support Europe's goal of becoming, in the coming years, a world leader in science and technology.

O.-W. Merten (ESACT)/H. Hermans (ACTIP)

**Ideas for improving our Newsletter**

Over the last month, I kicked off a discussion about how to improve our newsletters with the following suggestions:

1. Print an old, wizened article from previous meetings for the benefit of younger members
2. Have companies or research groups write about their technology platforms or research interests.
3. Expert reviews or opinions on a particular topic.
4. Solicit companies for sponsorship, awards...etc.

Some of the thoughts and suggestions tossed up by the committee members follow. In general, everyone (Alain, Christophe, Lisa, Otto, Stefanos, Francesc and Elisabeth commented) was positive about the first three points, but felt that the last one was the responsibility of the ESACT conference organising committee.

Specifically, **Christophe** suggested that it would be better to get research summaries from academia instead of industry as the latter might look like a company advertisement. However, he suggested that if a company were willing, it could take a major section of the newsletter and provide full sponsorship of the newsletter.

Otto was enthusiastic about such company sponsorship, and also liked the old wizened article idea.

**Stefanos** countered that getting industry sponsors may be attractive at first glance, but companies may not find this attractive if readership is not extensive. Also, we might become stretched looking for sponsors for each issue. Instead he

proposed that the team “blow a wind of change” to raise the profile and attractiveness of this newsletter, after which sponsorship would eventually be easier.

Finally, **Lisa** suggested that Fiona Godzman and Elisabeth Fraune, who are responsible for sponsorship, publicize ESACT and other bursaries for meetings, etc in the newsletter. She also suggested that old, wizened articles could be accompanied by a perspective/analysis – ie way ahead of its time or why we know better now. Lisa also suggested that we publish a list of noteworthy papers in animal cell technology nominated by ESACT members, similar to the Faculty of 1000 journal club [www.the-scientist.com/hotpapersarchive.htm#6](http://www.the-scientist.com/hotpapersarchive.htm#6) (see **HOT!! HOT!! HOT!!**).

Email your thoughts on these areas and contributions for future issues to [Steve.Oh@esact.org](mailto:Steve.Oh@esact.org). Thank you.

Steve Oh

23<sup>rd</sup> Aug. 2002

**Rethinking Cell Culture**

From commercial protein production to cell- and molecular-level modeling, cell culture is now one of the most enduring tools in science. Until recently, a fundamental limitation has persisted within the *in vitro* modeling applications – the sacrifice of either the indefinite lifespan of transformed cells or the modeling accuracy of primary cells. Here, my colleagues at CELLnTEC (Bern, Switzerland), Peter Girling and Dr. Eliane Mueller, give a brief look at recent advances in cell culture that have opened a range of new possibilities, providing investigators with the best of both worlds.

Lisa Hunt, Co-editor (eagerly awaiting your contributions for upcoming newsletters!)

### CELL CULTURE. WITHOUT COMPROMISE.

Beginning in the 1940's, there were several decades of tentative trial-and-error based discovery. In the last 15 years, many impressive developments in both media and culture techniques have been established in the cell culture industry. These new technologies have enabled many new cell types, *in vitro* model systems and specialised organotypic cultures to be established, culminating with the revolution in stem cell culture that began in the early 1990's.

However, despite these successes, investigators have often been forced to make an awkward sacrifice. Indefinite lifespan or optimal modeling – which has priority? On one hand, primary cultures offer the most accurate modeling of the *in vivo* situation, but without the ability for long-term growth, thus necessitating the continual establishment of new cultures. Conversely, long-term cultures can be obtained from transformed (immortalised or tumorigenic) cell types, but at the cost of modeling accuracy due to their altered genotypes.

Faced with this trade-off, scientists have always been forced to choose the best compromise for their specific application. This has left many to contend with the vagaries of a transformed cell line's altered genotype or phenotype, when for instance a long-term transformed culture was required for a stable transfection. For example, the HaCaT human keratinocyte cell line is one of many commonly used in *in vitro* models, despite its documented phenotypic and genotypic abnormalities (e.g. it is p53<sup>-/-</sup>).

Recent advances in the *in vitro* culture and trans-differentiation of stem cells have now provided new hope that such compromises will soon no longer be required. Several cell types have now been cultured, seemingly without transformation, well beyond the common threshold of 20 doublings where many cell types typically reach senescence using traditional culture techniques. A particularly impressive example was reported by Jiang et al., where rat and mouse bone marrow progenitors were cultured for more than 120 doublings without loss of their ability for *in vitro* trans-differentiation into several mesodermal, endodermal and endothelial cell types.

Such long-term, untransformed, *in vitro* cultures have also been developed from a range of epithelial tissues (Caldelari et al.), and are now the focus of *CELLnTEC*, a new spin-off company based in Bern. Untransformed keratinocyte precursors have now been established from a range of species (including mouse and soon human) that have now survived more than 250 doublings in culture without any detected alteration. These cultures can be grown organotypically, are stably transfectable, and can be isolated from either wild-type or custom transgenic animals. See **Fig. 1**.

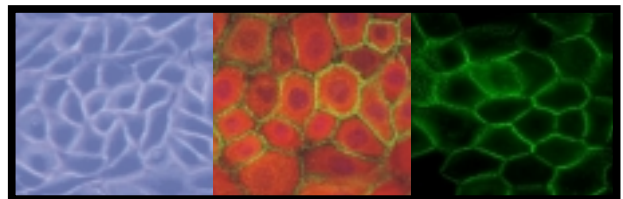


Fig. 1 shows a long-term mouse keratinocyte culture (left) stained for keratins 18, 5 & 6 (red) and desmoglein 3 (green) (middle), and stained for plakoglobin (green) (right).

The appearance of this new generation of cell cultures is now building momentum. Once complete for a full range of cell

types, concerns over the accuracy with which transformed cell lines model the *in vivo* situation will be drastically reduced. Stable, long-term cultures with all the modeling accuracy of primary cultures spell the end of ongoing re-isolation of new cultures from experimental animals, and hopefully the beginning of a new era of *in vitro* studies where artefacts associated with genetic transformation are no longer a significant source of inconsistent results.

In summary, remember next time you need an *in vitro* model system; you may no longer need to compromise modeling accuracy to get an indefinite lifespan!

#### **Peter Girling**

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#### **Dr Eliane Müller**

Institute for Animal Pathology, University  
of Bern, Switzerland.

#### References:

- Jiang et al. 2002. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature advanced online publication, 20 June 2002.  
Caldelari et al. 2000. Long-Term Culture of Murine Epidermal Keratinocytes. J. Invest. Dermatol., 114:1064-1065

For further information on the previous (and inaugural!) rethinking cell culture article, "Basal Culture Media," contact:

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## Stem Cell Updates

Science reported in August, that 14 centres claim that they possess human embryonic stem cell lines. These are described in the table below. Of these however, only Wisconsin Alumni Research Foundation (**WARF**) in Washington, USA and Embryonic Stem Cell International (**ESI**), Singapore have distributed 57 and 30 batches of hES cells respectively to researchers and collaborators. This is far more than all the other institutes combined, which have either not characterized the cell lines or are providing cells to limited numbers of collaborators.

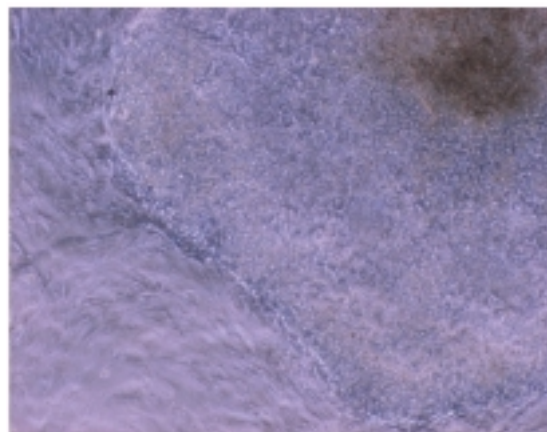


Fig. 2 Human embryonic stem cell colony growing on elongated "feeder" cells.

It is hoped that, with the increased availability of these cell lines, research on these most "potent" of cell lines will move ahead rapidly. However, there are many difficult issues of cell culture, for example hES grow very slowly and in tight clusters, on mouse feeder cell layers (Fig.2). Novel cell culture technologies will thus have to be developed to overcome the current laborious culture methods. And perhaps someday, ES like cells will be made without derivation from embryos?

Steve Oh

29<sup>th</sup> Aug. 2002.

Table 1. Institutions claiming human embryonic stem cell lines

Institutions	Listed	Now available
Arcos / CyThera – seeking NIH infrastructure award	9	0
BresaGen – 1 cell line supplied to collaborators. NIH infrastructure award	4	1
<b>ES Cell International</b> – have sent cells to 30 groups so far, 10 in the US NIH infrastructure award	6	4
Geron – Has WARF's 5 cell lines & 2 subclones. Supplying only NIH collaborators	7	0
Karolinska Institute, Sweden – Lines are frozen. NIH award	6	0
Maria Biotech, S. Korea – cells available to collaborators only	3	3
Pochon CHA University, S. Korea – still characterizing cell lines	2	0
Reliance Life Sciences, India – None fully characterized	7	0
Seoul National University, S. Korea – plans worldwide distribution	1	1
Tata Institute, India – None fully characterized	3	0
Technion University, Israel – Distributing to collaborators only	4	3
UCSF, USA – Plans to distribute 1 line. NIH award	2	0
University of Goteborg, Sweden – 1 paper under review, plans collaboration with US researchers.	19	3
<b>WARF</b> – Has executed agreements with 90 researchers and shipped 57 batches of 1 cell line. Plans to ship all 5 by Jan.	5	1

2003.

### Legal Framework on Human Tissue Engineered Products

We would like to draw your attention to the above consultation sent to ESACT by the European Commission Research Directorate-General. Please send your comments directly to [ENTR-TISSUE-ENGINEERING@cec.eu.int](mailto:ENTR-TISSUE-ENGINEERING@cec.eu.int) by 30 Sep 2002.

*Enterprise Europe, Brussels, 4 July 2002 - The Enterprise DG invites comments on the need for, and possible content of, a Community legal framework on human tissue engineered products. Please visit this website for more detailed information:-*

[http://europa.eu.int/comm/enterprise/medical\\_devices/consult\\_tissue\\_engineer.htm](http://europa.eu.int/comm/enterprise/medical_devices/consult_tissue_engineer.htm)

*Tissue engineering is new, fast-developing and largely unregulated. It aims to restore, maintain or improve the function of human tissues or organs. It differs from standard therapies in that tissue engineered products become integrated within the patient, affording a potentially permanent and specific cure for disease, injury or impairment. Typical applications are orthopaedic prostheses (bones), cardiovascular prostheses (heart valves, blood vessels, arteries), neurological tissue repair, skin repair, muscle repair, liver or pancreas regeneration or prosthesis, and prosthesis for the urinary tract.*

*At present, neither the development of knowledge and technology nor the potential risks are fully foreseeable. Potential benefits for patients are, however, thought to be huge. The challenge for the regulators is to enable patients to gain rapid access to new and*

*highly promising types of products, under optimal safety and quality conditions.*

*The Commission's Scientific Committee on Medicinal Products and Medical Devices<sup>(1)</sup>, feels that while tissue engineered products bear some similarities to both medical devices and pharmaceuticals (e.g. cell therapy products); they differ sufficiently from both to justify a specific regulatory system.*

*Lacking an EU-wide legal framework, Member States have begun taking national measures, disparities among which could prevent patients from having equal access to tissue-engineered products across the Union, and undermine confidence in these products.*

*Tissue engineering companies, too, need a clear legal framework to provide certainty as to the laws protecting their investments and activities.*

*The potential worldwide market for tissue engineered products is estimated at almost Euro 100 billion per annum (source: Pittsburgh Tissue Engineering Initiative).*

*European Commission Research Directorate General:*

*Gwennaël Joliff-Botrel (Directorate F - Life Sciences: health research)*

*Line Matthiessen-Guyader (Directorate E - Life Sciences: biotechnology, agricultural and food research) Open consultation on tissue engineering - to 30.9.2002.*

### **Report on the 8<sup>th</sup> Meeting on Cell Culture Engineering (Snowmass Village/CO, 1-6 of April 2002)**

This meeting was held at the Snowmass Conference Centre in the skiing domain of Aspen/CO, and my choice between skiing or science was governed by the quality of the presentations. Altogether, there were 4 keynote lectures, 30 normal lectures, 6 workshops, and two poster sessions with 150 posters. There were 320 participants, of which 2/3 were from the Americas, and 1/3 from overseas. Participants came from 113 companies and 53 academic institutions.

Of the 184 presentations, only the most interesting from my point of view will be described. The four keynote lectures were the following: **M.J. Shamblott** (Johns Hopkins Univ. Sch. Med., Baltimore/MD) gave a very interesting overview on human pluripotent stem cells and their potential use for regenerative medicine. Some spectacular examples, such as the treatment of type 1 diabetes of mice with embryoid body derived  $\beta$ -cells leading to insulin levels of about 1/10 of the normal values or the treatment of mice with paralysed hind limbs (paralysed due to a virus infection leading to neuronal death) leading to a rather successful improvement of their locomotion, were presented and indicated the bright future of this domain of biotechnology. **A. Varki** (Univ. California, San Diego) gave an excellent and animated overview on glycan diversity and differences between humans and the rest of the living world. This overview indicated evolutionary considerations and probable reasons for these diversities and tried to define the evolutionary advantages for the actual human being. **S.D. Patterson** (Celera Genomics Corp., Rockville/MD)

<sup>(1)</sup> Opinion of the State of the Art concerning tissue engineering, October 1, 2001.

gave a keynote lecture on the use of high efficiency proteomics for accelerating drug discovery. The keynote lecture by **R. Arathoon** (Genentech, San Francisco/CA) focused on some areas of enabling technologies that represent past, present, and future issues of the biotech industry: medium exchange technology for serum removal, the development and use of the heterobifunctional antibody technology.

Tissue therapeutics: **A.K. Smith** from Osiris therapeutics Inc. (Baltimore/MD) presented the use of human mesenchymal stem cells for tissue repair. In animal experiments, these cells appear to be non-immunogenic allowing the use of mismatched allogeneic mesenchymal stem cells. In preclinical studies, these cells successfully regenerated bone, meniscal tissue, and cardiac muscle without the need for immunosuppression. **M.V. Peshwa** from Dendreon Corp. (Seattle/WA) presented Dendreon's activities in the use of antigen presenting cell vaccines for cancer immunotherapy. By using a serum-free method to expand immature precursor antigen presenting cells from peripheral blood (without the addition of exogenous cytokines, but apparently via cell interactions), mononuclear cells are matured and tumor antigen loaded and then infused into the patients after quality control. Over 1000 clinical lots of these cells have been used to treat more than 300 patients with various cancers. **M. Mayer-Proschel** (Univ. Rochester/NY) presented the human glial progenitor cell line A2B5+, which can be amplified and differentiated into astrocytes and oligodendrocytes when different factors are added to the culture medium. An interesting observation was that two different types of oligodendrocytes were observed. When mitogens were used, the cytoplasm was in

an overall reduced state, and the cells proliferated better; whereas, when the cells were cultivated in the presence of differentiation factors, the cytoplasm was in a more oxidized state and the cells differentiated. These cells were much more sensitive to apoptosis than cells with a reduced cytoplasm.

DNA vaccines: **M.P. Calos** (Stanford Univ. Sch. Med., Stanford/CA) showed that by high pressure injection into the tail vein of mice of a plasmid coding for the human factor IX gene and the attB (a recognition site of the integrase of the  $\phi$ C31 phage) together with a plasmid carrying the integrase gene led to a maximal transduction efficiency of 40% of the liver cells. The expression of the gene was equivalent to the expression of the natural gene and persisted for more than 6 months. This integration is possible because a lot of "pseudo" attB sites are present in the genome of humans and mice. **D.S. Allison** (ICOS Corp. Bothell/WA) presented comparative data on the use of different promoters (CMV, human EF-1 $\alpha$  promoter) and the Chinese Hamster EF-1 $\alpha$  (CHEF1) transcription control sequence. Producer CHO clones which were transfected with plasmids containing the expressed gene under the control of CHEF1 produced 5 to 20 times higher titers and were more stable than those clones in which the gene was under the control of CMV, human EF-1 $\alpha$ , or SV40 promoters. This observation is also valid for cells of human and mouse origin.

**B.J. Potts** (Genentech, San Francisco/CA) gave an overview on adventitious viruses, and presented some new viruses, the circoviruses, which recently became of more concern because of their resistance against inactivation. Other recent viruses of concern are reoviruses, bovine polyoma

virus, the parvovirus B19, and the Cache Valley Virus. By the way, many potentially dangerous viruses are not mentioned in the 9CER document. SIV sequences in plasmid vaccines are equally a concern. **N.R. Cashman** (Univ. Toronto/Canada) gave an overview on prions as adventitious agents. He stated that the *in vitro* infection of cells via the use of contaminated materials is a possibility, because, for instance, human lymphocytes contain 5000-10000 PrP<sup>c</sup> molecules per cell. In principle, all cells express these proteins, indicating that the biological security might be increased when PrP<sup>c</sup> knock-out cell lines could be used for biotechnological applications. **J.A. Lewis** (Merck and Co., Inc., West Point/PA) presented the problems of safety testing of biologicals and, in particular, of viral vaccines. The overall testing (in vivo and in vitro tests) have a maximal duration of 52 days, and viral vaccines can only be evaluated after inactivation of these viruses before testing for adventitious viral agents.

Glycosylation: **W.R. Gombotz** (Immunex Corp., Seattle/WA) presented the studies on the glycosylation and C-terminal processing of recombinant human Flt3 ligand expressed by CHO cells. This protein has 3 Cys-Cys bonds, 2 N-linked, and 5 O-linked glycosylation sites. Produced under serum-free conditions, this protein shows less C-terminal cleavage, more double N-linked species, and more sialylation of the N-linked glycans than that produced in serum-containing medium. **M.S. Sinacore** (Wyeth BioPharma, Andover/MA) presented glycosylation and posttranslational modification issues concerning the production of recombinant P-selectin-Ig molecule in CHO cells. For correct glycosylation, it was necessary to co-express both the core 2 GlcNAc transferase and the fucosyltransferase

enzymes, necessary for building the fucosylated core-2 oligosaccharide of the sialylated Lewis<sup>x</sup> (SLe<sup>x</sup>) structure bound to the Thr<sup>16</sup>. This co-expression was an efficient strategy to promote synthesis of SLe<sup>x</sup> - bearing core-2 type O-linked oligosaccharide of the rPSGL-Ig. In addition, it was necessary to increase the cells' capacity to sialylate N-linked glycosaccharides as well as the sulfation of N-terminal tyrosines (Tyr<sup>5</sup>, Tyr<sup>7</sup>, Tyr<sup>10</sup>). This was achieved by adding 10 mM of N-acetylmannosamine to the medium for a better sialylation and by optimizing the cysteine and Mg<sup>++</sup> concentrations. Optimal levels were 2 mM of cysteine and 15 mM of MgSO<sub>4</sub>. The sulfation of the N-terminal Tyr could be increased from 78.4% under standard medium conditions to 91.2%. **R.A. Taticek** from Genentech (South San Francisco/CA) presented a glyco-optimisation study for improving the structure of monoclonal antibodies produced by rCHO cells. In order to get rid of acidic variants, no galactose should be used in the medium, although this sugar source was optimal for getting a high product yield. To obtain correct C-terminal lys-processing, bovine peptone had to be replaced by porcine peptone.

Viral vectors: **R.J. Samulski** (Univ. North Carolina, Chapel Hill/NC) gave a very interesting overview on the development of the different AAV serotypes and their potential applications. Their approach consisted in the use of rep2 - capx constructs, by always using the ITR of AAV2 for the construction of the rAAV plasmid. No packaging cell line development is done in his lab. In addition, he presented studies for optimizing the tropism of these vectors for different targets by conjointly using unmodified and modified cap-constructs. **S. Lai** from

GenVec Inc. (Gaithersburg/MD) presented a new 293 based packaging cell line for the production of adenoviral vectors for gene therapy purposes. As they are using E1/E4 deleted adenoviral vectors, and as there are no overlapping sequences between the E1 and the E4 sequence, these 293/ORF6 cells do not produce RCAs. In order to avoid negative effects of the ORF6 sequence of the E4 region of the adenovirus on cell growth, it is under regulation of a  $Zn^{++}$  inducible sheep metalothionine promoter. For safety reasons, these packaging cell lines were adapted to grow in protein free media, in which they grow in suspension. They can be grown in different scaleable reactor systems. The downstream processing, which consists of concentration/diafiltration, filtration, 2 column chromatographies, a gel filtration, and a final filtration step, has an overall recovery of 54%.  $6 \times 10^{13}$  PU/l can be produced in a reactor system, the lowest dose for the treatment of a solid tumor is  $4 \times 10^9$  PU. **L. Xie** from Merck Res. Labs. (West Point/PA) presented the optimisation of a production process of adenoviral vectors for use as HIV vaccine. Using the PERC6 cells, they found that optimal cell growth was achieved in a pH range of 7.0 to 7.7, whereas the pH for optimal vector production ranged between 7.0 and 7.4. The optimal vector production temperature ranged between 35 and 37.5°C. When the cells were grown at 33°C for 16 days before infection, the highest vector production was obtained at temperatures ranging between 35 and 37°C 48 h PI. The PERC6 cells are resistant to aeration rates of 0.05 vvm, however, after infection, they become very sensitive towards sparging and Pluronic F68 has to be added for protection. The process was scaled up to a reactor size of 300 l; however, there should

not be any problem to scale to a production size of 2000 l.

Monoclonal antibody technologies: **S. Sonderhoff** (Abgenix Biopharma Inc., Vancouver/Canada) presented the XenoMouse and XenoMax Technologies. By using mice which were knocked out for mouse Igs but engineered for the production human IgGs, IgMs, and IgDs, monoclonal antibodies with completely humanized peptide-sequences can be produced using the classical hybridoma technology. These antibodies can be produced with  $\kappa$  or  $\lambda$  light chains. XenoMax Technology is 10-100 times more efficient for identifying and getting monoclonal antibodies against rare antigens. **B.G. Turner** (Abbott Bioresearch Center, Worcester/MA) presented the manufacturing process development for D2E7, a recombinant human antibody to tumor necrosis factor produced using serum-free CHO cells. **D. Stark** from Genentech (Vacaville/CA) presented the new large scale antibody manufacturing facility in Vacaville (size: 12000 l).

Cell physiology and metabolism: **A.E. Morris** from Immunex Corp. (Seattle/WA) compared the expression of rec. genes in COS-1 and CHO/E5 cells. Whereas the overexpression of protein kinase Ba (PKBa) improved the expression of recombinant proteins (example: SEAP) and eliminated the need for serum in the expression medium for the COS-1 system, a similar effect was observed for CHO cells when ssMek was overexpressed. Such CHO clones grow in serum-free media free of growth factors or protein hydrolysates in suspension. The overexpression of PKBa in CHO cells had no positive effect. **W.P.C. Stemmer** (Maxygen, Inc., Redwood City/CA) presented a very interesting talk

on molecular breeding of genes, pathways by DNA shuffling, and the application to cell culture engineering. The examples (most of them published in Nature Biotechnology, Nature Genetics, PNAS, or similar journals) range from the development of a multivalent dengue virus vaccine by epitope shuffling to the increase of the shear resistance of retroviral vectors (improvement by a factor of 60-1000 fold). **L. Qu** (Protein Design Labs, Inc., Plymouth/MN) optimized the fed-batch production process for monoclonal antibodies. Due to the over-abundance of nutrients (glucose and amino acids), accumulation of lactate and ammonia may adversely affect culture performance. Although the use of feeding strategies of amino acids, glutamine, and glucose can lead to production levels of up to 1 g/l of monoclonal antibody, this approach worked only for about 50% of the cells. For the other cells, the addition of cocktails of vitamins and trace elements (which ones were not indicated) led to a higher OUR and therefore to a more oxidized metabolism. The spiking of this cocktail to the feed medium led directly to a peak in the O<sub>2</sub> consumption rate. It also led to reduced lactate production, culture osmolarity and an improved antibody yield by a factor of 2-8, depending on the cell line used.

Otto-Wilhelm Merten, Evry, 20.4.02

### Conference Report: 224<sup>th</sup> ACS National Meeting, Boston

Division of Biochemical Technology,  
August 18-22, 2002

Prepared by: Dr. Victor Wong, Singapore-MIT Alliance, National University of Singapore. [smawongv@nus.edu.sg](mailto:smawongv@nus.edu.sg)

The 5 day conference had multiple concurrent sessions covering a wide range of areas. In total, there were close to 230 oral and 97 poster presentations, including 3 plenary lectures given by the recipients of the 3 awards presented during the meeting: the Perlman, the Marvin J. Johnson and the Elmer Gaden Awards. This report will focus on presentations in 3 areas: Advances in Cell Culture Process Development, Bioinformatics, Genomics and Proteomics and Combinatorial and High-Throughput Approaches to Bioprocess Development.

With the increasing availability of high-throughput platforms for global analyses of DNA, RNA and protein levels, a number of presentations focused on the effects of culture parameters on the genomic and proteomic response of industrially important cell lines. For instance, **Marcela Gatti** from Prof Hu's group at University of Minnesota presented some preliminary results from their study on the effect of sodium butyrate on gene expression of recombinant mammalian cells. They compared the hybridization patterns of CHO and mouse hybridoma cells onto mouse cDNA microarrays and found similar trends. However, a number of genes showed opposite expression patterns between the two species (e.g. a sialyltransferase, was up-regulated in hybridomas but not in CHO). Nevertheless, they concluded that cross species hybridization of CHO cells on mouse arrays could be used to study CHO expression profiles.

**Dhinakar Kompala** from University of Colorado also studied the genomic and proteomic expression pattern of CHO cells. They investigated the effect of dexamethasone (a glucocorticoid analog) on the gene expression of CHO cells

containing the glucocorticoid-inducible mouse mammary tumor virus (MMTV) promoter. A suppression subtractive hybridization (SSH) technique was used to monitor the differential gene expression. However, it was reported that the SSH technique resulted in inconsistent gene expression patterns for each run.

While these studies illustrate the potential of global analyses of gene and protein expression of industrial cell lines to understand complex cellular pathways, an impressive example of the integration of genomic, proteomic and bioinformatic analyses of a systematically perturbed metabolic network was given by the speaker at this year's Perlman Memorial Lecture, **Leroy Hood**, from University of Washington. Using a "systems biology" approach, he explained how a map of the interaction network governing a cellular response could be constructed through iterations of perturbations and global measurements. He elegantly illustrated this approach with the galactose metabolism system in yeast (also appeared in *Science*, 292:929-934, 2001)..

On a more "conventional" bioprocessing front, **Jongchan Lee** from University of Minnesota described a process where hybridoma cells were grown in a fed-batch/continuous culture with on-line measurement of oxygen uptake rate, turbidity and inlet gas composition. Cultivation was initiated in fed-batch mode with low glucose control. Switching from fed-batch to continuous mode during the cultivation period enabled deficient nutrients to replenish and/or inhibitory compounds to wash out. In addition, nutrient feed and culture dilution was decoupled by using two independent feed streams (a balanced salt solution stream

and a concentrated nutrient solution stream). The feed nutrient concentration and dilution rate were manipulated to maintain cell and nutrient concentrations at constant levels. The culture was then switched back to fed-batch mode, which resulted in a higher cell concentration.

**Dr. A. Cayli** from Boehringer Ingelheim also described a fed-batch process on a recombinant CHO cell line producing an antibody. A feeding strategy that limits both the glucose and glutamine concentration was used, resulting in high cell and antibody concentrations (1.6 g/L). However, in spite of the high product yield, Cayli highlighted some concerns regarding the implementation of substrate limitation for large-scale cell culture processes (15000 L). Firstly, implementing an on-line substrate limitation control increases the risk of contamination. Secondly, small variations in substrate concentration could mean that substrate concentration can be zero during the fermentation. This might result in apoptosis and the risk of losing the entire 15000L run. He concluded that the additional economic benefit from substrate limitation control has to be weighed against the additional risk of this strategy for each new process. In general, achieving high yield through media optimization was preferred as it is a safer strategy.

**Jens Vogel** of Bayer described some components of a set of platform technologies developed at Bayer which can be assembled to form a continuous manufacturing process for protein drugs. For example, the use of sterile bags to bank cells, instead of the traditional cryovials, enables the initial thawing of 50-100ml of cell culture directly into a 2L bioreactor. This significantly reduces the inoculum expansion time and offers greater control

of the inoculum expansion process. Bayer is evaluating the use of continuous annular chromatography in their process, but raised concern over non-homogeneity in resin packing and the problem of maintaining sterility in the rotating seals. In addition, their focus on automation and robotization highlights their desire to minimize process variability and reduce labor cost.

**Tina Etcheverry** of Genentech talked about a multi-factorial design approach to measure the influence of several cell culture parameters on cell growth, metabolism and product quality on different antibody-producing CHO cells. The two key product quality characteristics for antibodies were glycosylation and charge-heterogeneity profiles. The extent of galactosylation of the Fc region is important for effector function and ADCC. Changes in the charge-heterogeneity profile, such as deamidation and acidic variants, can potentially reduce the antigen binding affinity of the antibody. By grouping the various process parameters and carrying out a factorial design experiment for each group, they were able to identify media (especially glutamine) and temperature as factors having adverse effects on galactosylation, even though they appear to boost product titer. In addition, culture time and pH showed interactive effects on charge heterogeneity.

**Yuan Xu** presented Genentech's strategy for characterizing the genetic consistency of production cell lines, in line with new ICH<sup>2</sup> guidelines. A battery of nucleic acid and protein analytical methods were adopted. Yuan concluded that protein analysis of each product lot was a more

convincing method than nucleic acid testing for verifying the correct product coding sequence of the expression construct in the production cell lines.

Many of the presenters acknowledged that a high-throughput approach to bioprocess development was necessary to investigate the large number of variables that can potentially impact product yield and quality in a cell culture process. For example, running multiple bioreactors to investigate a large number of process parameters may be impractical and costly. Several novel technologies that can facilitate parallel experimentation of multiple conditions were presented during the conference.

For example, **Brett Schreyer** from BioProcessors Corp described a micro-fluidic cell culture device, which can potentially grow 200 µL of cell culture in a controlled environment similar to that of a conventional bioreactor. By integrating micro-fluidics, miniturization, automation and software control, this system has the potential for providing highly parallel and automated experimentation. Brett presented data showing that the growth characteristics for CHO, NS0 and hybridoma cells in the prototype device were comparable to that in micro-well plates. At present, the prototype device sits inside a conventional incubator, but it is envisaged that a commercial model will have its own temperature control. It is likely that liquid loss through evaporation may be a significant problem at such minute volumes, although the presenter argued that it can be minimized by maintaining a high humidity. The final version may only be commercially available in 2 to 3 years.

**Peter Harms** from Govind Rao's laboratory at University of Maryland

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<sup>2</sup> International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

presented a similar technology utilizing a disposable 24 well plate as a parallel bioreactor system with online optical sensors to measure DO, pH and cell mass. Agitation, aeration and temperature control were also provided. To date, the device has only been used to grow E. coli cultures, running 4 wells concurrently. The microplate sits inside a laminar flow-hood to maintain sterility. However, it is not clear if the numerous wires and connections feeding to the system would disrupt the laminar flow and compromise sterility. In its present form, the microplate is open to atmosphere, hence, liquid loss through evaporation may become significant for runs over an extended period typical in mammalian cell culture. This system is adapted from the cuvette-based microbioreactor described in **Govind Rao's** paper in *Biotechnology and Bioengineering* (72: 346-352, 2001) for which he was presented with the Elmer Gaden award during the ACS meeting.

### **HOT!!!! HOT!!!! HOT!!!!**

Share YOUR opinion on what is hot now in animal cell technology. In the next issue of the ESACT newsletter, we will publish a list of hot papers nominated by you, our highly-qualified and faithful readers.

Simple: Nominate a hot paper in animal cell technology by sending a **concise** (not more than a few sentences) summary of the significant contribution to [lisa@cellntec.com](mailto:lisa@cellntec.com) by December 15, 2002.

Depending on the success of this first call for your participation, HOT!!!! HOT!!!! HOT!!!! could become a regular feature of the ESACT newsletter.

### **Joke Corner**

News break! Scientists are initiating clinical trials of the first gene therapy product that prevents erectile dysfunction and named it **Elongation Factor I**. Its common name is **ViGoRous**. This gene, when combined with an *enhancer* element, also satisfies women.

Dwarfs worldwide have embarked on sequencing their genes and called it the **"Human Gnome"** project. They are currently highering new staff.

Mice have taken over animal facilities in a respected institution and gone on hunger strike. They complained of the boring pellet and water rations.

Submit good jokes to [Steve.Oh@esact.org](mailto:Steve.Oh@esact.org)

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The usual plea to keep me informed of address/Email/telephone changes, and to the many that have not informed me of their Email address the information that they are missing out on the regular Email Bulletins sent to members. Also a reminder that members 3 years or more in arrears of their subscription are deemed to have resigned.

With Best Wishes

Bryan Griffiths

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