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Editorial

Dear Readers,

January and has come and gone with speed and it's again time for the ESACT newsletter. Scurrying for information, we have been able to pull together a selection of contributions that we hope will whet your appetite.

This issue has contributions from Ferruccio Messi, Juergen Lehman, Mohammed Al-Rubeai, Otto Merten, Jerry Tong, Lisa and myself covering a diverse range of topics.

We would like to reiterate that contributions are very welcome from fellow members in future issues.

Chief Editor, Steve Oh

Rethinking Cell Culture

Despite our best efforts to be thoroughly scientific, animal cell culture is pervaded by ancient practices and beliefs. Many basic protocols were developed, in a thoroughly scientific way, years ago. Take, for example, the detailed studies of freezing and thawing made by ESACT secretary Bryan Griffiths *et al.* in the 1970's. We all appreciate and benefit daily from the work put into developing these standard protocols. With all due respect, cell culture practices can and should be questioned and tested - now. New developments and new knowledge could contribute to an improvement of the dear, familiar standard protocols.

Sometimes ancient practices are perpetuated for lack of a better option. However, sometimes these practices are continued even when a proven improvement exists – either because we are simply not aware of the new development, or because we humans are set in our ways and resistant to change. The goal of this new column, Rethinking Cell Culture, is to encourage discussion and the exchange of ideas

about aspects of cell culture that are so basic that we take them for granted. In this edition, Feruccio Messi suggests a new paradigm for basal media.

Your own personal experience, knowledge and questions are welcome – and necessary!!!!

Lisa Hunt, Co-editor

BASAL CULTURE MEDIA

ESACT has been the witness of the technological revolution concerning *in vitro* animal cell culture since the seventies. New insights about structures, features, and cultivation techniques of mammalian cells have been facilitating the development of new strategies for the generation of biologicals and research concepts based on mass cultivation. Cultured mammalian cells at industrial scale are synthesizing many of today's -and tomorrow's - genetically engineered products, ranging from vaccines to immunologicals, enzymes and hormones.

From a technological point of view, one main goal has been recently attained: the recognition of the nutritional and physicochemical requirements of cultured cells, and the application of fully synthetic, protein and peptide-free minimal culture media allowing wider control over the complex cellular growth requirements. This achievement opens new prospects for biological studies, i.e. the understanding of the cell metabolism and regulation. Furthermore, the design of a proper environment to fit the cellular needs during cultivation provides the extension of cell culture applications in terms of more rational synthesis and expression of recombinant genes, thus creating the prerequisites to further developments in bioprocessing.

There is no doubt that the above accomplishment will show a number of benefits to *in vitro* cell culture, but the path leading to progress of the current state-of-the-art technology remains steep.

Cell culture still represents a quite tortuous/tricky field of application to many operators. Every day, cells cease to proliferate, do not behave as expected, or simply quit for no evident reason. While the explanation for such failures is not necessarily obvious, difficulties in maintaining mammalian cells in culture environments free of exogeneous proteins or complex additives often arise from a lack of understanding of the roles played by the individual medium components. Unfortunately, some experimental cell culture work performed in the past decades has been misunderstood, thus leading to the following paradox in cell culture.

The most widely used basal culture media in the biotech industry today were developed long before some of the most relevant animal cell lines for industrial application (such as CHO and hybridomas) were either selected or deposited in internationally recognized cell banks. As a consequence, these cells have been grown for years with unsuitable nutrient mixtures that were previously conceived for completely different purposes. Obviously, it is difficult to achieve cell proliferation in such sub-optimal culture environments and, therefore - here comes the trouble -, cell biologists began to think that the cells had an essential requirement for exogeneously added proteins, hydrolysates, growth factors, complex lipid mixtures etc. to successfully proliferate *in vitro*.

The most widely used basal medium for the industrial cultivation of CHO cells in the last two decades, the nutrient mixture DMEM/F12, contains 52 ingredients and must be supplemented with nutrient extensions to sustain both cell growth and recombinant protein expression. Ironically, medium supplementation is necessary to overcome poor cell growth due to the presence of growth

inhibitors and nutritional imbalances in the same basal medium. This paradox is proven by the fact that CHO cells proliferating in protein- and peptide-free minimal media consisting of less than 50 low-molecular ingredients of highest purity can be conveniently used for developing a complete industrial bioprocess^{1,2}. As a conclusion, the cells do not require so-called complex medium supplements to grow; they just need improved basal nutrient mixtures capable of satisfying their nutritional requirements.

At present, two more aspects make the industrial application of protein and peptide-free, minimal cell culture media more difficult. The first one is related to the poor availability of personnel with technical skills for growing mammalian cells under chemically defined culture conditions. It is still difficult for the cell culture community to understand how different the cultivation of cells in minimal media can be when compared to cultivation of cells in complex culture environments. When it comes to cell harvesting, it is not rare to find operators centrifuging mammalian cells for over 10 minutes at 1000 g or cultivating cells in culture vessels exposed to light for days. Said manipulations can be harmless for serum-dependent cells, but might be pretty deadly to cells growing in protein- and peptide-free minimal media. We will stop here to avoid a tedious list of technical disasters occurring in cell culture labs all over the world every day. The second aspect rendering the application of fully synthetic minimal media difficult is related to their manufacturing procedure. Powdered media preparations used to represent the most comfortable solution at the time of complex medium supplementation, but currently show their limits in terms of growth-supporting capacity in protein and peptide-free cell culture. On the other hand, alternative manufacturing methods such as fluid bed granulation still have to prove their consistency when it comes to the manufacturing of large lots of product. So far, only liquid preparations - particularly liquid concentrates - have been able to prove their suitability for producing *any* required medium

formulation at *any* lot size - ranging from 1 to over 1,000,000 liters per single lot. Current progress on manufacturing and packaging technology has eliminated the typical disadvantages of liquid preparations once represented by logistic problems, thus making liquid concentrates ready for a more extensive application in cell culture. Last, but not least, media preparation by companies devoted to the manufacturing of bulk liquid solutions (for applications other than *in vitro* cell culture) might contribute to open up the quite conservative cell culture media market by offering excellent product technologies and large manufacturing capacities at low cost.

The development and the correct application of fully synthetic, protein and peptide-free minimal culture media opens interesting perspectives for research and industrial purposes. This approach allows a more extended control and modulation of the cellular metabolism, thus creating the basis for a new area of development in cell biology: the rational design of media for cell phenotypes. Welcome to tomorrow.

Feruccio Messi

1) Messi, F. (1991). "*Tuning of structure and function of Chinese Hamster Ovary (CHO) cells by systematic design of defined micro-environments*". Ph.D.-Thesis No 9559, Swiss Federal Institute of Technology, Zurich, Switzerland.

2) Zang, M., Trautmann, H., Gandor, C., Messi, F., Asselbergs, F., Leist, C., Fiechter, A. and Reiser, J. (1995). "*Production of recombinant proteins in Chinese Hamster Ovary cells using a protein-free cell culture medium*". *Bio/Technol.* 13, 389-392.

UK Government OKs Research Involving Embryonic Stem Cells

The UK government has removed the last legal barrier to stem cell research using human embryos, paving the way for the UK to take the lead in this field as the only country with a precise regulatory framework governing such research.

The Human Fertilization and Embryology Authority, the agency that regulates the research, said it had granted the first two licenses to scientists at Edinburgh University and King's College, London, allowing them to culture stem cell lines from embryos left over from *in vitro* fertilization (IVF) treatments.

The two main academic funding bodies, the Medical Research Council (MRC) and the charity The Wellcome Trust, are expected to announce grants worth several million pounds over the next few months for embryonic stem cell research. The MRC also immediately called for tenders from laboratories to set up and run an independent UK national stem cell bank.

Parliament voted last year to allow therapeutic cloning but said no research permits should be granted until a House of Lords committee, headed by the Bishop of Oxford, Richard Harries, completed a review of the scientific and ethical issues.

In its report last week the committee rejected the argument that advances in research on adult stem cells mean research on embryonic stem cells is unnecessary. It said embryos left over from IVF should be used in preference to embryos cloned for the purpose, but that cloning should be allowed where "there is a demonstrable and exceptional need which cannot be met in other ways."

The committee said it did not believe this approval would create a slippery slope leading to reproductive cloning.

The UK BioIndustry Association said these conclusions reinforced the pre-eminence of the UK. "[It] sends a positive message that the UK is the right place for this research," said the chief executive, Crispin Kirkman.

The Wellcome Trust also welcomed the report. "The House of Lords have endorsed UK research into the therapeutic potential of stem cells, which is already creating a precedent for the rest of the world," said Mike Dexter, director of the trust.

"Scientists can now get on with finding treatments for life-threatening diseases such as Parkinson's, diabetes and cancer, thanks to this common sense report."

George Radda, chief executive of the MRC, said the national stem cell bank will be set up as a matter of urgency. "Such a bank will allow researchers to explore this enormous potential in a controlled environment." The council is developing a set of principles to cover the ethical, legal and regulatory issues associated with the bank, and is devising donor information leaflets and consent forms.

The MRC also is hopeful that the decision will attract stem cell researchers to the UK from abroad.

Although the go-ahead is expected to lead to an explosion in stem cell research, it will have no direct effect on Europe's only publicly quoted stem cell therapy company, ReNeuron Ltd., of Guildford, Surrey, because its cell lines are derived from fetal stem cells.

Published March 6, 2002 in Bioworld by Nuala Moran

Biomedical Science Initiatives in Singapore

Singapore's Biomedical Sciences International Advisory Council (IAC) concluded its 4th meeting in London today. The Council comprises 13 renowned scientists and academic leaders from Europe, USA and Australia and is chaired by Sir Richard Sykes, Rector of Imperial College, London, and Chairman of Glaxo SmithKline plc, and co-chaired by Dr Sydney Brenner, Distinguished Professor, Salk Institute for Biological Studies, USA.

The Council was updated on the progress of Singapore's Biomedical Sciences initiatives and endorsed the formation of the new Institute of Bioengineering (IBE), which will be funded and supervised by Singapore's Agency for Science, Technology and Research (A*STAR).

The IBE will be established this year to capitalise on Singapore's existing strengths in engineering and medical sciences, and will synergise the work of other research groups in Singapore. The new Institute will focus on research in tissue and stem cell engineering, biomaterials and scaffolds, medical devices and delivery systems. It will also be actively involved in new technology platforms such as computational biology, imaging and analysis of biological systems and nanotechnology. When fully established, IBE could be home to about 250 research scientists and engineers.

The IBE will be co-located with the Genome Institute of Singapore, the Bioprocessing Technology Centre, the BioInformatics Institute, and the merged Institute of Molecular & Cell Biology (IMCB) and the Institute of Molecular Agrobiolgy (IMA) at the Biopolis, a new research park in Singapore dedicated to the Biomedical Sciences. The first phase of the Biopolis is targeted to be ready by mid-2003.

The IAC was appointed in Year 2000 to advise the Singapore Government on its plans to develop Singapore into a Biomedical Sciences centre in Asia, and to grow the Biomedical Sciences into one of the four key pillars of economic growth, alongside the electronics, chemicals and engineering industries. Since the IAC's first meeting in September 2000, subsequent meetings have been held in the US and in Singapore. For this 4th meeting in London, 12 members of the Council were present. They discussed a range of issues including public research and infrastructure development.

Sir Richard Sykes, Chairman of the IAC, said, "Singapore has made tremendous progress in developing the Biomedical Sciences industry in

a relatively short span of time. Many initiatives in research, education and industry development have been implemented, which serve as a positive testimony of the nation's keen determination to position Singapore as a key player in the international arena for Biomedical Sciences. The Council is greatly impressed with Singapore's cohesive and integrated approach towards developing the Biomedical Sciences and is confident that Singapore is headed for success in this field. We look forward to more exciting developments to come in the future."

Dr Sydney Brenner, Co-Chairman of the IAC, added, "The new Institute of Bioengineering at the Biopolis forms a critical piece of Singapore's R&D infrastructure. Together with the setting up of the Genome Institute of Singapore, the BioInformatics Institute, the Biomedical Grid and the Singapore Tissue Network, this will ensure that there is a strong science and innovation base to support modern biomedical discovery and development activities. These institutes will form a strong research foundation for Singapore to attract new investments in private Biomedical Sciences research and industrial activities."

Information on the Biomedical Sciences industry in Singapore is available at <http://www.biomed-singapore.com>.

Press release on the 6th Mar. 2002 by Agency for Science Technology & Research

**Dissertations from the Institute of
Cell Culture Technology,****University of Bielefeld, Germany****1) Development and Use of a Peptide
Affinity Matrix for Selective Protein
Purification by Nicole Ameskamp**

The aim of the investigations was to design an optimized chromatographic matrix for the use in conventional packed bed and integrated expanded bed adsorption. Utilizing small peptide ligands, a high performance affinity resin was developed, which combines the selectivity of common affinity materials like Protein A with the chemical stability of group-specific ligands such as ion exchangers. The studies were carried out with a monoclonal antibody against the coagulation factor VIII, which was used as a model protein.

The ligand screening focused on combinatorial libraries of hexapeptides, which were produced by spot synthesis technology on cellulose membranes. A dual positional scanning strategy enabled a fast and reliable selection of a suitable affinity peptide, rendering unnecessary the material- and time-consuming overall synthesis and testing of the existing 64 million variants of possible hexapeptides.

In the second step the selected sequence was coupled to a NHS-activated base matrix. In order to optimize the ligand accessibility and to insure the same binding conditions as in spot synthesis, linkage had to be done by an orientated attachment via the C-terminus of the peptide. For this purpose three strategies with varying modifications of the base material and the peptide ligand were applied. The resulting affinity matrices showed no differences in their chromatographic performance and proved a very high selectivity towards the monoclonal antibody.

Packed bed experiments were carried out with small 1 ml columns whereas the expanded bed

process was performed with 70 ml of a special EBA prototype matrix. In both modes the target protein could be successfully purified with yields of more than 97%. SDS-PAGE analyses showed excellent depletion of contaminating proteins like the media supplements albumin (500 mg/L), transferrin, and insulin (each 10 mg/ml). The concentrated IgG eluate exhibited no remaining impurities of host cell DNA, which were determined by means of a fluorescent intercalating dye with a detection limit of 10 ng/ml. Applying an affinity-purified solution of the antibody (approx. 640 mg IgG/L) in frontal adsorption analyses the dynamic binding capacity was found to be more than 25 mg IgG/ml gel in packed bed mode.

Using the peptide affinity matrix in expanded bed adsorption enabled a robust integrative downstream process with excellent clarification, concentration and purification results of high reproducibility. Portions of about 1000 ml of cell culture harvest, containing approx. 50 mg IgG, were successfully processed in less than 90 minutes. Unlike e.g. ion exchangers the peptide matrix showed no interactions with the biomass, which would cause an unstable performance and low product yields. Furthermore, the high stability of the peptide ligands enabled a treatment with 0.5 M NaOH insuring an easy cleaning-in-place protocol, which is usually practicable for robust matrices only.

Due to the selective binding properties and the high chemical stability, the new peptide resin proved to be a powerful alternative for common chromatographic materials especially for the use in integrative expanded bed adsorption. Additionally, the investigations showed that combinatorial spot synthesis is an easy and universal method for the selection of suitable peptide ligands. Utilized with oriented ligand coupling chemistry, it offers reliable technology for a product-specific development of high capacity peptide affinity matrices.

**2) Hypothermic, non-freezing storage of
hepatocytes under pH- and pO₂-controlled**

conditions for application in bioartificial liver support systems, by Kai Iding

Background: Bioartificial liver support using immobilized hepatocytes in a hollow fiber bioreactor is the most encouraging approach to overcome the problem of donor organ shortage in liver transplantation medicine. One of the main challenges is the continuous supply of hepatocytes at any time a medical application is needed (*'just in time supply'*). Demand is unpredictable and therefore stocks of a certain amount of hepatocytes have to be built up.

Accordingly, cryopreservation is an already well established method to store animal cells over years but it requires the use of partially toxic preservation additives like DMSO. Hence, we investigated the behaviour of hepatocytes under hypothermic conditions (4°C) to obviate the necessity of such substances.

Materials and Methods: One human and two murine hepatocyte cell lines were each expanded on macroporous microcarriers using a conventional cultivation medium with 5% FCS in a pH- and pO₂-controlled bubble-free aerated 1-L-bioreactor. Subsequently, the bioreactor was chilled and cell viability was monitored by trypan-blue exclusion method for approximately one week. Afterwards the temperature was adjusted to normal conditions.

Results: Best results were achieved with the murine line HepT when the cells were cooled down from 37°C to 4°C under hyperoxic (i.e. oxygen-rich) conditions and subsequently stored at 4°C under anoxic (i.e. oxygen-free) conditions. The moderate decrease of viable cells (rate: 2%/per day) enabled a satisfactory storage time of more than one week. Additionally, growth rate of the hepatocytes after hypothermic storage corresponded to the normal growth rate. Unfortunately, both C3A (human) and K105 (murine) died rapidly within one or two days of hypothermic storage independent from oxygen concentration at 4°C.

Conclusion: Hypothermic, non-freezing storage is an alternative to cryopreservation for a comparatively short period of time. Nevertheless, conditions have to be estimated for each hepatocyte cell line.

Report on the 9th Meeting of the European Society of Gene Therapy (Antalya, Turkey, 2-4 of Nov. 2001)

The meeting was organised in the Resort Dedeman Hotel of Antalya and the negative effect of the terrorist attack on the USA on the 11th of September on the participation was visible. Two weeks before the meeting about 400 people had already registered but only about 300 people were present and many many Americans were absent. All sessions except the satellite sessions took place. About 40% of all speakers had to be replaced (due to absence) by speakers of the same group or by other talks as the nominated speakers were not always present. It is evident that the task of the organizers was very difficult and despite these problems, the meeting was quite good although some would have preferred to have more US speakers because they are often considered to be ahead of us (at least in some parts of the gene therapy field).

The meeting was organised in plenary sessions, parallel sessions and posters sessions. Some highlights will be presented, which are selected from the point of view of an engineer rather than a person active in the fundamentals of gene therapy.

J.M. Heard (Institute Pasteur, Paris/F) presented an approach for the treatment of β -thalassemia through the stimulation of fetal like erythropoiesis via the stimulation of the synthesis of γ chain of globin. The gene transfer by using rAAV into muscles lead to a tetracycline inducible expression of globin and thus to an expression of the BFU-E pool and CFU-E pool (erythroid progenitors)

M. Collins (Univ. Coll. London/UK) reported on the transduction of monocytes and dendritic cells by HIV-1 based LV vectors (VSV-G enveloped) indicating that such cells can only be efficiently transduced after a certain differentiation had been accomplished. Without this differentiation the preintegration-complex was not transported into the nucleus. These vectors (LV or MLV based) were produced under serum free conditions with transiently transfected 293T cells.

A.M. Douar (Genethon III, Evry/F) reported on factors influencing gene transfer efficiency of AAV vectors.

J. Lisziewicz (Research Inst. for Genetic and Human Therapy, Washington/DC) presented results for genetic immunisation studies to control retroviral (RV) replication in macaques. By applying Dermavir_{SHIV} with a new composition of a plasmid DNA with mannosylated PEI to skin, Langerhans cells (1000 c/mm²) can be efficiently transfected. These DNA expressing Langerhans cells migrate to the T cell areas of the draining lymph nodes interdigitate as dendritic cells and present DNA derived antigen to T cells. Using such an approach the application of 0.025 mg of DNA on the skin resulted in approximately 20,000 gene expressing dendritic cells in non-human primates. Using this approach in form of a fixed scheduled structured treatment interruption approach (STI-HAART) for reduced viral load in SIV infected macaques leads to a considerable reduction from about 4.3×10^6 to finally 200 copies/ml. This indicates that STI-HAART in combination with Dermavir might be efficient for controlling HIV replication in HIV positive individuals. A typical application will be a prophylactic one.

D. von Laer (G. Speyer Haus, Frankfurt/G) presented a gene therapy approach for the treatment of HIV infection by transducing T cells for expressing a membrane anchored peptide derived from the 2nd heptade repeat of gp41 inhibiting viral entry in cell lines and primary T cells for all tested HIV variants.

Y. Barrandon (Ecole Normale Supérieure, Paris/F) presented an overview on multipotent stem cells and skin morphogenesis.

W. Uckert (Max Delbrück Centre for Molecular Medicine and Gene Therapy, Berlin/D) presented a study on the efficiency of transducing human haematopoietic cells using oncoretroviral and lentiviral vectors. As a conclusion, they found out that the use of MPSV-LTR was much more efficient than the use of MLV-LTR for the expression of the Transgene (difference of about 2 logs). The most efficient env protein for transduction was the 10A1 env.

N. Taylor (IGMM, CNRS-UMR5535, Montpellier/F) reported on the biology of neonatal and adult T-cells. Even when using LV vectors naive cells cannot be transduced. This is only possible after induction of these cells and after the first cell cycle has been achieved, indicating that efficient transduction is dependent on the activation/maturation state and not on the cell cycle programme of these cells. In any case, human primary T cells are not electroporable nor transfectable, only MLV/LV based gene transfer is possible.

The two invited speakers of the cell process session were **M. Wisher (Bioreliance, Stirling/UK)** and **J.M. Guillaume (Aventis Pharma, Vitry sur Seine/F)** reporting on quality control of viral vectors for gene therapy and production and characterization of adenovirus for gene therapy, respectively. With respect to the production of vectors for gene therapy, the rigorous quality control testing of cell banks, raw materials etc, is of utmost importance due to absence (in most of the cases) of downstream processing. Cell bank testing has shown that 3.5% of them were mycoplasma positive, 2.5% positive for bovine derived viruses and 2.2% positive in MAP testing. For instance, in research departments up to 9.5% of the cell lines are positive for

mycoplasmas. Testing for the absence of adventitious viruses is very important because they can be introduced via FCS (PI virus, BVDV, reovirus 1-3), trypsin (porcine parvovirus, porcine circovirus), but also via the use of raw material, such as amino acids or glucose (MVM). A newly discovered FCS derived virus is, for instance, the cache valley virus, a bunyavirus, signifying that a rigorous quality control is mandatory.

As a general rule, from 2003 onwards, all biotech products have to be produced under GMP conditions, the manufacturing facilities have to be inspected and the clinical material has to be released by a qualified person.

J.M. Guillaume described the experience of **Aventis Pharma (Gencell)** in the field of the production of AdV vectors. They have developed a

serum free large-scale process for AdV vectors production. For optimising the process, it was monitored by on-line HPLC in order to follow the release of viral particles. After optimisation 1-2 x 10¹¹ vp/ml were obtained in a 75 l perfusion reactor, 50 l of harvest were generally collected, whereby 400 l of medium (in total) were used. The specific productivity ranged between 35,000 and 100,000 vp/c independent of the transgene. The downstream processing leads to a yield of 64% (clarification, concentration, IEC, concentration/diafiltration), the viral vector are formulated at 10¹² vp/ml in a TRIS buffer. Safety tests were performed with respect to the presence/absence of replication competent Adenoviruses (RCAs) and prions. Whereas RCAs were found in 293 based productions, only non-conventional RCAs were found in the productions done with PERC6 cells. The limits for normal RCAs were < 1RCA/3x10¹⁰ particles or < 1RCA/10⁹ infectious particles. In preparations of > 1,9 x 10¹⁰ particles about 30% were RCA positive. The prion assessment of 293 and PERC6 cells revealed that both cells had the normal wild type PrP gene, that they were heterozygous at the met/val codon 129, that PrP was present at normal concentrations

and that no PrP^{sc} was present. However, no in vivo assessment was performed. With request to vector production, both cells produced 1.1x10¹¹ particles at day 7, the cell densities were 2x10⁶ c/ml at day 3 (PERC6 cells) or at day 4 (293 cells).

H. Schneider (University of Erlangen, Erlangen/D) used ex vivo AdV transduced syngeneic fibroblasts as vehicles for the production of IGF-1 or BMP-2 in order to induce chondrogenesis for cartilage repair. The advantage of this approach is the localized activity of the produced and secreted growth factors, the duration of gene expression (up to 28 days) no spread of the vector and the avoidance of both, the strong cellular immune response and the formation of antibodies against AdV elicited following direct vector application. The duration of 4 weeks of transgene expression is enough for the onset of cartilage regeneration.

G. Dickson (Univ. London/UK) presented the development of micro-dystrophin viral vector and synthetic oligo-nucleotide approaches for gene therapy of muscular dystrophy. It seems that in all cases (expression of mini or micro-dystrophin) immuno-suppression will be necessary.

S. Braun (Transgene, Strasbourg/F) presented the therapy approach developed by Transgene (use of plasmid DNA: safer than viral vectors, skeletal muscles can be transduced in vivo, the vector is not immunogenic). In the MDX mouse model, anti-dystrophin antibodies appear 7-28 days after administration. The actual phase 1 study makes

use of a plasmid concentration of 200 µg or 600 µg/patient (the plasmid was formulated in isotonic phosphate buffer, pH 7.4). The perspective is an intra-arterial delivery, which could be shown to be functional in macaque. This approach will be tested in GRMD dogs.

D. Wells (Department Neuromuscular Diseases, London/UK) presented an optimised method for the electro transfer to muscles. Using a hyaluronidase pre-treatment the electroporation voltage could be reduced to 175V/cm leading to reduce muscle damage. Using LacZ as transgene 30-40% of fibres were transduced, with respect to dystrophy only 10-15% of the fibres were positive.

S.J. Russell (Mayo Foundation, Rochester, MN/USA) presented the advantages of an altered biodistribution of targeted lentiviral vectors: reduced vector load target only the tissues to be targeted. The modification of the env protein (introduction of peptides into the amphotropic env protein) led to a reduction in the vectors titres (about 10^5 lentiviral particles per ml were obtained).

M. Havenga (Crucell, Leiden/NL) presented improved adenoviral vectors for gene therapy and vaccinations. The derived characteristics for vaccination are high antigen presenting cell tropism, high skeletal muscle tropism, and high potent T cell activation. For efficiency reasons, the AdV vectors should not be neutralized after administration. By comparing fibre proteins of different adenoviruses it became obvious that F35 equipped adenovirus had a high affinity for human muscle biopsies, hepatocytes, and LPS matured dendritic cells, as well as a very high T cell activation. Ad5F35 efficiently transduced mature and naive dendritic cells and monocytes, even at a low MOI. F35 vectors are the least prone to neutralisation.

Dr. Salima Hacein-Bey-Abina (INSERM, U429, Hop. Necker, Paris/F) gave an interesting follow up of the clinical study for treating x-linked SCID defects (due to γ deficiency).

About 75% of the 224 announced posters were exhibited/presented.

Despite the adverse situation, the meeting was interesting.

O.-W. Merten, Evry, 13.12.01

Report on the Second European Meeting on Cell Engineering

Costa Brava, 25 - 28 October 2001

Sponsored by: The European Commission, Life Sciences

Meeting Chairmen: M. Al-Rubeai, Birmingham, H. Hauser, GBF, U. Schlokot, Baxter

The Second European Meeting on Cell Engineering took place in a charming hotel on the Costa Brava, Spain, from 25th to 28th October, 2001. About 80 participants and invited speakers created a familial atmosphere in a gorgeous surrounding, with exciting discussions among small groups of scientists lasting until very late at night. The emphasis of the meeting was to present and discuss the latest research results, to review the current state of animal cell engineering and to bring together junior and senior scientists from academia and industry alike. While this topic used to be of interest mostly to researchers involved in pharmaceutical protein production, it has become of equal importance for the development and production of gene and cell therapeutics, vaccines and tissue engineering. Accordingly, participants were experts in the respective areas, from industry, academia and other public research institutions.

Advances in basic cloning technologies were reported by **F. Stewart** (University of Dresden), based on homologous recombination systems cloning in *E. coli* by means of bacteriophage-derived *in vivo* recombination systems. In particular, the ET technology allows the cloning of large DNA fragments and offers a means to precisely manipulate eukaryotic chromosomal fragments (BACs) in bacterial cells without the need for unique restriction sites.

J. Unsinger (GBF, Braunschweig) reported the development of a new autoregulatory expression cassette that allows regulated gene expression. The cassette can be integrated into conventional, retro- or adenoviral vectors or into a new hybrid retro/adenoviral vector, which combines advantages of virus stability, high infectivity, highly regulated and stable expression characteristics.

M. Fussenegger (ETH Zurich) presented alternative, antibiotic-based regulated gene expression systems based on streptogramins, erythromycin and other macrolides. The new systems can be used in parallel with the established tetracycline regulated system in the same cells, thus allowing the independent regulation of multiple genes. These regulatory systems can also be employed as sensitive biosensors for macrolides and streptogramins, for example in applications of monitoring food quality.

In the last decade, progress in protein glycan analysis and the isolation of relevant glycosyl transferase genes now allows systematic cell engineering to achieve defined glycoprotein structures. The approximation of protein glycosylation of expressed protein molecules according to authenticity and thus meeting the needs of pharmaceutical application is a significant step forward in this technology. **L. Krummen** (Genentech, Inc., San Francisco) and **H. Conradt** (GBF, Braunschweig) gave impressive examples of these applications and showed strategies on how to redesign existing cell lines to produce such glycoproteins with improved *in vivo* properties.

A highlight of the meeting was the presentation of **R. Kaufman** (Howard Hughes Medical Institute, Ann Arbor) who enrolled two pathways of stress response to the expression of unfolded proteins in the ER. A complex network of post-translational activities, RNA splicing, transcription and translation is responsible for this type of stress response. The presentation emphasized the importance of understanding and integrating these steps.

Comprehensive overviews on cell systems and their genetic manipulation were given by **F. Wurm** (EPFL, Lausanne) and **S. Weiss** (PerBioScience, Belgium) on stable as well as large scale transient expression systems in mammalian cells and baculovirus infected insect cells, respectively.

Developments in the cultivation and visualisation of proteins in cardiomyocytes were presented by **Hans Eppenberger** (ETH, Zürich). Heart tissue is prone to irreparable damage during a heart attack, since these cells stop dividing shortly after birth. Professor Eppenberger showed that heterologous proteins of the contractile apparatus are integrated specifically into the appropriate sites. He also presented techniques for *in vitro* cultivation of these cells and for efficient genetic modification. By using Sindbis virus vectors, a recombinant gene was expressed in up to 80% of cultured cardiomyocytes.

Expression of recombinant growth factors is a key step in the engineering of mesenchymal stem cells towards bone tissue engineering. In contrast to cardiomyocytes, the culture of primary human osteoprogenitor cells is possible. **R. Oreffo** (Univ. of Southampton, UK) used recombinant BMP-2 producing recombinant adenoviruses to engineer these cells and to populate biodegradable 3-D scaffolds for potential orthopaedic use.

The problem of cell death in bioreactors was dealt with by **F. Godia** (University of Barcelona), **J. van de Goor** (Genentech, San Francisco) and **T. Sauerwald** (Johns Hopkins University, Baltimore). They identified the major origin of cell death in hybridoma, CHO and 293 cells as apoptosis. Typically, activation of caspases was found to be increased, mainly caspases 3 and 9. Using chemical inhibitors and the expression of anti-apoptotic proteins, apoptosis in bioreactors can be inhibited. Using this technology, a significant improvement of cell survival in

fermenters and after thawing of frozen cells was achieved.

Vaccination is a specific form of Cell Engineering.

While cell technologists try to avoid apoptosis, the opposite is the objective in vaccination. Here, apoptosis of normal cells expressing specific antigens as a result of vaccination leads to a strong enhancement of protective and therapeutic immune

responses. Apoptotic cell fragments are taken up by professional antigen presenting cells to present these antigens to the adoptive immune system. Alphavirus vectors and DNA immunisation examples had been employed in the vaccination strategies described by **P. Liljeström** (Karolinska Institute, Stockholm) and **E. Rollman** (Karolinska Institute, Stockholm), respectively.

During an extended session, several speakers demonstrated advances in tools and technologies for animal cell biotechnology. **K. Herrenknecht** (Evotec OAI, Hamburg) provided an overview on cell based Ultra High Throughput Screening in submicroliter format using automated confocal microscopy and sophisticated image analysis, allowing for the screening of 100,000 components per day. **T. Ryll** (Abgenix, Fremont, CA) reported about a recently emerging powerful approach to routinely produce human type antibodies in mice containing the human Ig-locus (Xenomouse) and the isolation of immunoglobulin genes encoding rare antibody species by direct cloning from relevant B-cells which obviates the need for labour intensive 'humanised' cloning and expression procedure by 'conventional' routes. The isolation of single cells secreting defined amounts of a certain recombinant protein by a gel micro drop method was introduced by **Y. Akselband** (One Cell Systems, Cambridge, MA). In this method, single cells are embedded in a droplet, the secreted protein is captured, labelled and the droplets are isolated by fluorescence cytometry. This method may have a tremendous beneficial

impact in the search for high yield expressers as well as stable clones. **C. Kemp** (Kemp Biotechnologies, Frederick MD) reported on the production of selenomethionine and biotinylated protein production in baculovirus infected insect cells. These technologies are relevant for structural research and protein isolation, respectively.

In addition, 19 posters were presented throughout the meeting and could be discussed with the authors during a scheduled poster session. Upon careful evaluation by a jury chaired by Prof. **Randall Kaufman** (Howard Hughes Medical Institute, Ann Arbor, MI) consisting of 3 internationally renowned scientists. The three most innovative and scientifically sound posters; presented by **F. Kramberger** (Baxter, Orth-Donau), **L. Rosenbrier** (University of Manchester, UK) and **P. Muller** (GBF, Braunschweig) each received an award.

Overall, the most impressive progress was apparent in the visualisation of molecules in single living cells and single cell sorting based on their products, the high degree of automation and the ever increasing use of bioinformatics to handle the overwhelming flood of data from genome analysis and expression profiling. Significant advances were presented in culture and expansion of cells and tissues, understanding and manipulation of apoptosis, cell growth, differentiation and cellular stress responses, development of novel cloning techniques, viral vectors and regulated gene expression systems. Key issues and pathways to potentially overcome limitations in the overproduction of recombinant proteins of interest in tissue culture cells were identified. These limitations currently represent a major impediment to a more effective production of biopharmaceuticals. Better understanding of these limitations will hopefully lead to the establishment of additional cell lines of improved suitability and value than are currently available. There is still a need for more reliable manipulation of mammalian cells

to obtain cell lines with predefined characteristics. Methods to allow for more common use of homologous recombination in cell lines are still missing. This deficit is not only reflected by the extensive amount of work and time required to achieve high and stable expression for each new product, but also by the lengthy process to obtain the approval for new production processes by the regulatory authorities.

In summary, this meeting demonstrated promising and powerful approaches of how to overcome a number of critical limitations to current cell culture systems.

Mohammed Al-Rubeai, Dec. 2001

Useful Research Phrases **(Jeremy Tong)**

Phrase: It has long been known...

Means: I didn't look up the original reference.

P: Typical results are shown.

M: The best results are shown.

P: These results will be shown in a subsequent report.

M: I might get around to this sometime, if I'm pushed.

P: It is generally believed that...

M: A couple of other people think so too.

P: It is clear that much additional work will be required before complete understanding of the phenomenon occurs.

M: I don't understand it.

P: It is hoped that this study will stimulate further investigation in this field.

M: This is a lousy paper, but so are all the others on this miserable topic.

P: Thanks are due to Joe Blotz for assistance with the experiment and Sally Frink for valuable discussion.

M: Blotz did the work and Frink explained to me what it meant.

P: A statistically oriented projection of the significance of these findings...

M: Wild guess.

New Members

We would like to welcome to ESACT the following new members;

Jennifer Bond (Imperial College, UK);
Victoria Chesters (GlaxoSmithKline, UK)
Alan Cullen (The Agency, USA); Magnus
Doverskag (Biovitrum, Sweden); Nicolas
Haverlange (Henogen SA, Belgium)
Morten Praestegaard (Bioimage A/S,
Denmark); Juliet Varley (Imperial College,
UK); Finn Wiberg (Symphogen A/S,
Denmark); Mon-Han Wu (Imperial
College, UK)

ESACT Secretariat

Subscriptions (now due for 2002). The official currency of ESACT is now the Euro, however as the main ESACT Accounts are in the UK all subscription payments (by credit cards) will be deducted in £ sterling. A reminder of fees;

Members working in Europe (Full).....20 Euros (= £13)

Members working outside Europe (Associate)...30 Euros (= £18)

Members are expected to re-new their membership automatically every January as reminders are not sent out. When company invoices are needed for payment please contact me individually

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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